

Hot water extractable carbon in whole soil and particle-size fractions isolated from soils under contrasting land-use treatments

Denis Curtin^{A,*} , Mike H. Beare^A  and Weiwen Qiu^A 

For full list of author affiliations and declarations see end of paper

***Correspondence to:**

Denis Curtin
The New Zealand Institute for Plant and Food Research Limited, Private Bag 4704, Christchurch, New Zealand
Email: denis.curtin@plantandfood.co.nz

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ABSTRACT

Context. Understanding the dynamics of soil organic matter (SOM) requires that it be divided into fractions with contrasting behaviour. **Aim.** Using soils from a field trial with a diverse set of treatments (pasture to arable conversion, ex-pasture soil maintained fallow for 13 years and arable cropping with contrasting tillage treatments), we evaluated two approaches to characterise SOM: particle-size fractionation and hot water extraction. **Methods.** Soils were separated into four size fractions, including the >50 µm fraction (particulate organic matter, considered the most labile fraction) and the <5 µm fraction (stabilised C associated with clay particles). Hot water extractable C (HWC; 80°C for 16 h) was determined on whole soil and on the isolated size fractions. **Key results.** Whereas total soil C stocks (0–25 cm) declined by an average of 14% under arable cropping and by 23% under continuous fallow, the decline in HWC was much greater (31% under arable and 49% under bare fallow). A large part (>50%) of the C lost under cropping and bare fallow was derived from the clay fraction, which was also the source of 59–77% of HWC. **Conclusions and Implications.** Our results indicate that hot water is specific for the labile component of SOM while SOM in size fractions is a composite of labile and stable components, the proportions of which may vary depending on land use and other factors. Ideally, data on quantity of SOM in size fractions should be complemented by information on its lability.

Keywords: hot water extraction, labile C, land use effects, long-term fallow, particle size fractionation, pasture, soil organic matter, tillage.

Introduction

Soil organic matter (SOM) is vital to the health and functioning of soils. It also plays a central role in the global cycles of C and nutrients, including nitrogen (Powlson 1993; Janzen 2004; Stockmann *et al.* 2013). The SOM comprises a heterogeneous mix of constituents, from rapidly decomposing plant residues to stable, persistent molecular structures. Knowledge of the composition of SOM is essential to understand the turnover of C and N in the soil–plant system.

Fractionation of organic matter based on particle size and solubility in (hot) water are two commonly used methodologies to obtain information on the proportions of labile and stable organic matter in soils. Association with the mineral matrix is a key mechanism stabilising SOM against decomposition (Dungait *et al.* 2012; Beare *et al.* 2014). It is well accepted that physical protection of SOM *via* sorption to the fine mineral fraction is more important in relation to SOM lability/bioavailability than is chemical recalcitrance (Kalbitz *et al.* 2005). In the size fractionation approach, organic matter recovered in the coarse fraction (sand particles >50 µm), referred to as particulate organic matter (POM), is regarded as the most labile fraction (Skjemstad *et al.* 2004; Poeplau and Don 2013). The sand-sized fraction is usually dominated by quartz and other low-surface area minerals that afford little protection against decomposition. The fine fractions (silt and

clay) contain phyllosilicate minerals and oxide/hydroxides of iron and aluminium that provide large surface area and numerous sites onto which organic matter may sorb and be stabilised.

A large number of physical fractionation schemes have been used to characterise SOM. Fractionation procedures can differ considerably in complexity, including the number of size fractions isolated. Some schemes may have four or more fractions (Poeplau *et al.* 2018), but a recent report concluded that separation of SOM into just two fractions, POM and mineral associated organic matter (MAOM), is adequate to understand and predict SOM dynamics (Lavallee *et al.* 2020). Ideally, the SOM fractions isolated by physical separation should be homogeneous and clearly distinct from each other (Smith *et al.* 2002). Increasing the number of size fractions is likely to result in isolation of SOM fractions that are more homogeneous in their composition and bioavailability. It is widely accepted that POM represents the most labile (bioavailable) size fraction, owing to its generally uncomplexed form and high turnover rate (Gregorich *et al.* 2006). Organic matter in the clay fraction may be more stable than that in the silt fraction (von Lützwow *et al.* 2006) and, thus, there may be justification for separating these two size fractions.

There is convincing evidence that organic matter extracted in hot water is labile (Ghani *et al.* 2003). Strong correlations have been demonstrated with several measures of labile SOM, including soil respiration in laboratory incubations (McNally *et al.* 2018), mineralisable N (Curtin *et al.* 2017b) and microbial biomass (Sparling *et al.* 1998). Hot water is a mild extractant and the proportion of total SOM extracted is relatively small. For example, 2–7.5% of soil organic C is typically removed in a 16-h hot water extraction at 80°C (Ghani *et al.* 2003; Curtin *et al.* 2006; Chantigny *et al.* 2010). Therefore, hot water will likely recover only a small proportion of total labile soil C in soils. The proportion of hot water extractable C (HWC) derived from different soil physical fractions is unknown, but it has been suggested that the most labile physical fraction, POM, has low water extractability (Lavallee *et al.* 2020). Thus, HWC and POM could provide conflicting information in relation to soil C lability in response to factors such as land use change or management perturbations. Understanding how physical fractions contribute to HWC is important to enable reconciliation of physical and chemical (extraction) approaches to SOM characterisation.

In this study we examined the effect of land-use change (including a pasture to arable conversion) and tillage practices on the particle-size distribution of C. The isolated size fractions were used to answer these questions: (1) what is the contribution of different size fractions to soil HWC? and (2) how does the hot water extractability of the C in particle size change in response to land management practices?

Materials and methods

Field experiment

Soil samples were collected in September 2013 from selected treatments in a field trial at Lincoln, Canterbury, New Zealand (43°40'3.5"S, 172°28'11.0"E). The trial, on a Wakanui silt loam (Udic Dystocrept), was established in November 2000 to examine the effects of tillage practices on SOM following conversion of long-term ryegrass (*Lolium perenne* L.)–white clover (*Trifolium repens* L.) pasture to arable cropping. The experiment had a split-plot design with tillage type (intensive, minimum or no tillage) as main-plot treatment and winter cover crop (plus or minus winter forage crops) as sub-plot treatment. The tillage treatments follow:

1. Intensive tillage: cultivation to ~20 cm using a mould-board plough, followed by secondary cultivation (one pass with a spring tined implement, followed by harrowing and rolling);
2. Minimum tillage: cultivation applied only to the top 10 cm using a disc implement, followed by secondary cultivation (harrowing and rolling);
3. No tillage: no soil cultivation, seeds direct drilled.

Treatments were replicated three times in an incomplete Latin square. Main plot size was 28 m × 18 m (sub-plots 28 m × 9 m). The tillage treatments were applied in both spring (prior to establishing the main crops) and autumn (before establishing the winter cover crops) using standard commercial equipment. All crops were sown using a Great Plains direct drill. The arable rotation of spring-sown crops included barley (6 years), wheat (2 years), peas (2 years) and ryegrass seed crops (2 years). Irrigation and fertiliser were applied to ensure that water and nutrients did not limit crop production. The winter cover crops included forage rape (8 years), winter cereals (wheat, oats and barley) and grass seed crops. Further trial details may be obtained from Fraser *et al.* (2013).

Replicated plots representing the original ryegrass–clover pasture (which had been undisturbed for ~14 years prior to trial initiation) were maintained within the trial design as a control treatment. Half of each pasture main plot was chemically fallowed (maintained plant-free using glyphosate herbicide; not cultivated or physically disturbed) throughout the experiment (i.e. permanent bare fallow). The pasture sub-plots were grazed using sheep (typically 10 times per year; 20 sheep per sub-plot). The bare fallow and pasture sub-plots were irrigated in summer (water application rate was the same as for the arable crops). Management (irrigation, fertiliser application and grazing) of the pasture plots remained essentially the same as before the trial. No fertiliser was applied to the fallow plots.

In total, samples were taken from 15 sub-plots, representing five treatments (i.e. treatments with arable crops

established using either intensive, minimum or no tillage; permanent pasture; and bare fallow). In the case of the arable cropping treatments, samples were taken from sub-plots where the main crops (spring sown) were followed by winter cover crops each year of the trial. Soil samples were collected by depth (0–7.5, 7.5–15 and 15–25 cm) using a 5-cm diameter stainless-steel corer at seven locations along a lengthwise transect in each sub-plot. Sub-samples from each depth increment within a plot were composited to form a bulk sample. The sampling depth of 25 cm was selected to exceed the depth of the deepest cultivation (i.e. ploughing to ~20 cm).

Laboratory methods

In the laboratory, the soils were weighed, sieved (<4 mm) and moisture content determined to enable calculation of bulk density. Sub-samples were air-dried for determination of total C and HWC and for size fractionation. Total C was measured using a LECO TruMac C/N analyser at a combustion temperature of 1250°C (LECO Corporation, St. Joseph, MI, USA). As the soils were acidic (pH 5–6) and free of inorganic C, all of the measured C can be assumed to be in organic form. The total stocks of C to 25 cm were calculated from soil C concentration and bulk density data.

The HWC was determined using the method of Ghani *et al.* (2003) with slight modifications. Briefly, soil samples (4 g) were extracted with 40 mL of deionised water on a temperature-controlled (80°C) water bath for 16 h, after which the soil slurries were centrifuged (2944g for 20 min) and the supernatants filtered through pre-leached filter papers (Whatman 42). Dissolved organic C in the hot water extracts was determined using a Total Organic Carbon Analyzer (Shimadzu TOC-V_{CSH}, Shimadzu Corp, Japan).

Particle-size fractionation was carried out after dispersing soil samples using an ultrasonic vibrator (30 g soil in 40 mL deionised water, 60 s sonication and power output 64 J s⁻¹). Preliminary studies showed that this sonication treatment was effective in dispersing these soils, while minimising fragmentation of coarse organic matter particles (Qiu *et al.* 2010). The sand fraction (>50 µm), including the POM, was

separated by passing the dispersed soil suspension through a 50-µm sieve. The <50 µm material was further fractionated (20–50, 5–20 and <5 µm) by gravity sedimentation (Gee and Or 2002). After separation, the sand- and silt-sized fractions (20–50 and 5–20 µm) were allowed to settle on a bench before siphoning off the excess water and air drying. Sufficient CaCl₂·2H₂O was added to the <5 µm suspensions to flocculate the clay particles. After settling, the water was siphoned off and the <5 µm fraction was air dried. Note: hereafter, the <5 µm fraction will be referred to as ‘clay’, even though it will contain some material (2–5 µm particles) usually regarded as fine silt-sized particles. The mean proportions [± standard deviation (s.d.)] of the size fractions follow: clay, 25 ± 2.5%; fine silt, 14 ± 1.7%; coarse silt, 25 ± 2.4%; and sand, 36 ± 4.9%.

Total C concentrations of the particle size fractions were determined using a LECO TruMac C/N analyser. The HWC in the fractions was determined by extracting 2 g samples in 40 mL of water for 16 h at 80°C, as described above for whole soils.

Statistical analyses

The ANOVA was performed using the R package ‘agricolae’ (ver. 4.0.5) (R Core Team (2018)). Least significant differences (l.s.d.) at *P* < 0.05, to detect difference among the treatments means, were calculated using the same R package. Linear regression analysis was used to quantify the relationships between measured variables.

Results

Total soil C

After 13 years of bare fallow, the soil C stock to 25 cm had decreased by 19 t ha⁻¹ (23%) compared with permanent pasture (Table 1). The top 7.5 cm layer accounted for half of the C lost under fallow with the 7.5–15 and 15–25 cm layers each accounting for approximately one-quarter of the total C loss. Losses of C under arable cropping were smaller

Table 1. Total C and hot water extractable C (HWC) in the 0–7.5, 7.5–15 and 15–25 cm sampling depths under permanent pasture, bare fallow and arable cropping using intensive tillage, minimum tillage and no tillage.

Treatment	Total soil C (g kg ⁻¹)			HWC (mg kg ⁻¹)			Soil C stock (t ha ⁻¹)	HWC stock (kg ha ⁻¹)
	0–7.5 cm	7.5–15 cm	15–25 cm	0–7.5 cm	7.5–15 cm	15–25 cm	0–25 cm	0–25 cm
Pasture	34.9	25.3	18.9	2213	1440	850	82.9	4608
Fallow	20.4	18.2	14.7	913	707	407	63.7	2360
Intensive tillage	22.0	21.8	19.9	1042	940	835	71.7	3152
Minimum tillage	27.8	23.4	15.0	1416	1044	564	73.6	3284
No tillage	27.0	20.0	15.0	1439	910	565	69.2	3171
l.s.d. (0.05%)		2.8			171		8.6	454

than under fallow (average decrease of 11 t C ha⁻¹), reflecting the contribution of crop residue inputs to maintaining soil C stocks. The individual tillage treatments affected the vertical distribution of soil C, i.e. intensive tillage homogenised the C concentration within the 25 cm layer, whereas soil C exhibited depth-stratification under the non-inversion tillage treatments (under no- and minimum-tillage, C concentration decreased with depth) (Table 1). However, tillage type did not significantly ($P > 0.05$) influence the total stock of C.

SOM size fractionation

Losses of soil C during the particle-size fractionation procedure were relatively small; recovery of soil C in the size fractions was $95.0 \pm 3.7\%$, comparable with that reported by Poeplau *et al.* (2018) for particle-size fractionation using ultrasonic dispersion. Carbon concentration (g C kg⁻¹ fraction) in the isolated size fractions was greatest in clay (58 ± 11.5 g kg⁻¹ clay), intermediate in fine silt (20 ± 6.4 g kg⁻¹ fine silt) and sand (6.4 ± 3.7 g kg⁻¹ sand) and least in the coarse silt (3 ± 1.3 g kg⁻¹ coarse silt) (data not shown). In the surface layer (0–7.5 cm) of pasture soil, 66% of the (recovered) C was in the clay fraction and 14% was POM-C (Fig. 1). The proportion of soil C in the clay fraction increased with increasing depth (to 75% in the 15–25 cm layer), whereas the proportion of POM-C decreased (to 7% in the 15–25 cm layer). In pasture soil, the proportions of soil C in the fine and coarse silt fractions changed little with depth; $14 \pm 1\%$ of soil C was recovered in the fine silt and $4 \pm 0.1\%$ in the coarse silt.

After 13 years under bare fallow, C in all four size fractions was significantly less than in the pasture soil (Table 2). The POM fraction showed the largest relative decrease (47% decrease in POM-C stock to 25 cm) and clay-C had the smallest relative decrease (clay-C stock to 25 cm decreased by 17%). However, clay-C was the largest of the measured SOM fractions, and accounted for more than half (52%) of the C lost from the top 25 cm during the 13-year bare

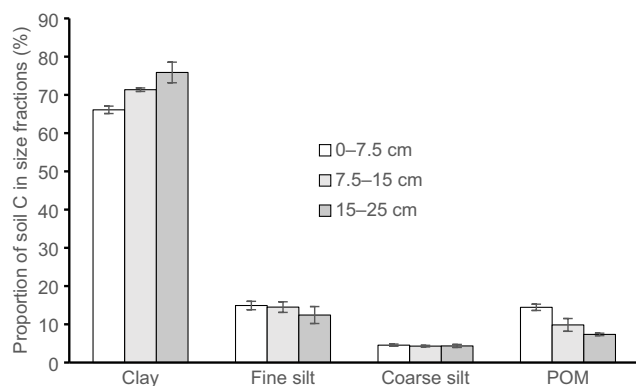


Fig. 1. Particle-size distribution of soil C in the 0–7.5, 7.5–15 and 15–25 cm layers of pasture soil. Bars represent ± 1 s.d. from the mean. POM, particulate organic matter.

Table 2. Total C stock in soil particle size fractions in the top 25 cm under different treatments at the end of the trial in 2013.

Treatment	C stock in size fraction (t ha ⁻¹)				
	>50 μ m	20–50 μ m	5–20 μ m	<5 μ m	Σ fractions
Pasture	8.5	3.5	11.1	55.8	79
Fallow	4.5	2.1	7.6	46.1	60
Intensive tillage	6.5	2.0	9.7	48.3	66
Minimum tillage	8.7	2.3	10.5	49.0	71
No tillage	7.7	3.0	8.9	46.9	67
I.s.d. ($P = 0.05$)	1.16	0.42	1.72	4.45	6.6

fallow period (compared with 22%, 7% and 18% for the POM, coarse silt and fine silt fractions, respectively; Table 3). The clay fraction was also the source of much of the C lost under the arable cropping rotation; this fraction accounted for between 60% (intensive tillage) and 83% (minimum tillage) of C lost from the top 25 cm during the 13 years of arable cropping. The POM-C fraction showed little change under arable cropping (particularly under low-disturbance tillage systems) suggesting that inputs of C in crop residues were sufficient to maintain POM-C stocks.

HWC in whole soil and particle-size fractions

The response of soil HWC to the management treatments paralleled that of total soil C, i.e. total stock of HWC (0–25 cm) was greatest under pasture, least under bare fallow and intermediate under arable cropping (Table 1). Soil HWC concentration declined with increasing depth. In all three sampling depths, the minimum and no-tillage treatments had closely similar values, whereas intensive tillage had lower near-surface (0–7.5 cm) values but higher values at depth (15–25 cm) compared with the two non-inversion tillage treatments. The total amount of HWC in the 0–25 cm layer did not differ ($P > 0.05$) between tillage treatments.

Across treatments and sampling depths, there was a strong linear relationship between HWC and total soil C (Fig. 2).

Table 3. Proportion (%) of C loss under bare fallow and tillage treatments attributable to different particle-size fractions relative to the long-term continuous pasture (control).

Treatment	Proportion of loss by fraction (%)			
	POM (>50 μ m)	Coarse silt (20–50 μ m)	Fine silt (5–20 μ m)	Clay (<5 μ m)
Fallow	22 $\pm$ 8	7 $\pm$ 0.5	18 $\pm$ 3	52 $\pm$ 7
Intensive tillage	16 $\pm$ 4	13 $\pm$ 2	11 $\pm$ 3	60 $\pm$ 2
Minimum tillage	–3 $\pm$ 6	14 $\pm$ 2	6 $\pm$ 8	83 $\pm$ 12
No tillage	5 $\pm$ 3	4 $\pm$ 0.5	18 $\pm$ 1	73 $\pm$ 3

POM, particulate organic matter.

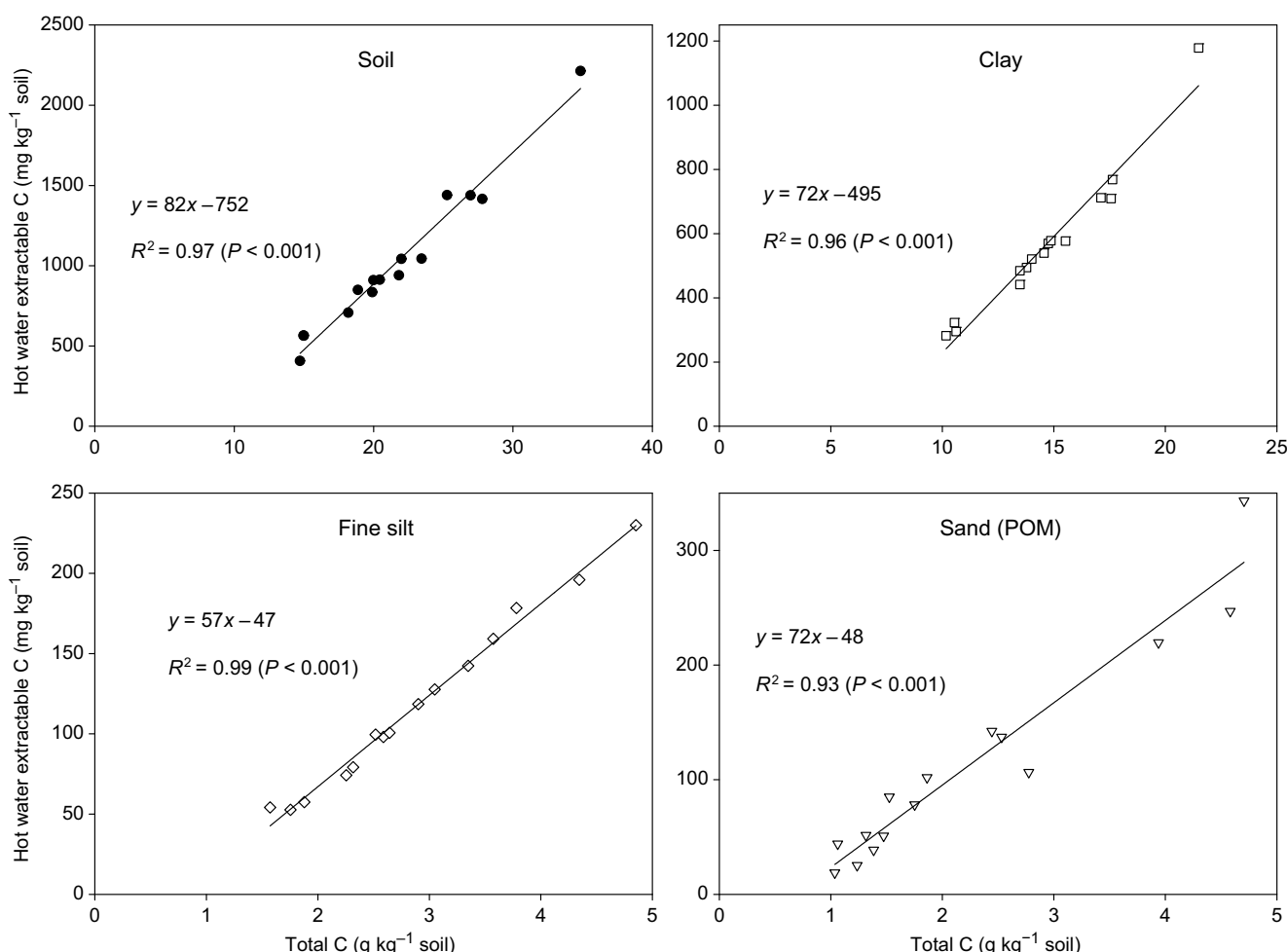


Fig. 2. Relationship between hot water extractable C and total C in whole soil and soil particle-size fractions (clay, fine silt and sand). Data represent different sampling depths (0–7.5, 7.5–15 and 15–25 cm) under permanent pasture, bare fallow and arable cropping using intensive tillage, minimum tillage and no-tillage. POM, particulate organic matter.

However, there was a highly significant intercept ($P < 0.001$), suggesting that part of the soil C stock did not release C to hot water (i.e. it was insoluble in hot water). The ‘insoluble C’ (intercept value on x-axis) was 9.6 ± 0.6 g kg⁻¹ soil. The HWC was more responsive to the treatments (and sampling depth) than was total soil C. For example, whereas total C in the top 25 cm of bare fallow soil declined by 23% relative to pasture soil, the decline in HWC was 49%. Under arable cropping, HWC declined by $31 \pm 1\%$ vs a decline in total C of $14 \pm 2\%$. The solubility of soil C in hot water (i.e. amount of C extracted in hot water per unit of total soil C in the 0–25 cm depth) was greater in pasture soil (55 mg HWC g⁻¹ soil C) than in cropped soil (45 ± 0.6 mg g⁻¹ soil C), with fallow soil exhibiting the lowest C solubility (37 mg g⁻¹ soil C).

Carbon extracted from the particle-size fractions using hot water amounted to $83 \pm 6\%$ of whole soil HWC. Recovery of HWC in the size fractions was less than that of total C ($95 \pm 3\%$), possibly indicating that the organic matter ‘lost’ during

the fractionation process included constituents with relatively high solubility in hot water. The clay fraction had the greatest concentration of HWC (expressed as mg kg⁻¹ fraction), followed by the fine silt (Table 4). Depending on treatment and depth of sampling, 59–77% of HWC (i.e. HWC recovered from all size fractions) was in the clay fraction. The fine silt fraction accounted for 12–17% of recovered HWC. The small contribution of this size fraction, which had a relatively high HWC concentration (mg HWC kg⁻¹ fine silt), reflects the fact that the mass proportion of fine silt was small (only $14 \pm 1.7\%$ of soil mass). The contribution of the POM fraction was variable (4–20% of soil HWC was in the POM fraction), depending on treatment (particularly low values in fallow POM) and depth of sampling, while on average only $5 \pm 1.5\%$ of recovered HWC was in the coarse silt. As with whole soil C solubility (described above), C solubility in the size fractions was usually greatest in the fractions isolated from pasture soil and least in fallow soil fractions (Table 5). For

Table 4. Hot water extractable C (HWC) in soil particle-size fractions of under different treatments and sampling depths.

Treatment	HWC in fraction (mg C kg ⁻¹ fraction)				Proportion of soil HWC in fraction (%)			
	>50 µm	20–50 µm	2–20 µm	<5 µm	>50 µm	20–50 µm	2–20 µm	<5 µm
0–7.5 cm								
Pasture	1040	389	1571	4357	19	5	13	64
Fallow	149	189	660	2143	7	6	13	74
Intensive	399	115	862	2354	16	3	15	66
Minimum	605	191	1325	2886	19	4	17	61
No tillage	646	284	1366	3036	20	6	15	59
I.s.d.	307	49	218	469	9.8	0.9	1.3	2.1
7.5–15 cm								
Pasture	427	279	1080	2860	12	6	14	68
Fallow	79	168	517	1815	4	7	12	77
Intensive	301	121	806	2177	13	4	15	68
Minimum	290	132	997	2345	12	4	17	67
No tillage	203	156	789	2250	11	5	13	71
I.s.d.	110	46	178	397	1.8	1.0	1.1	1.8
15–25 cm								
Pasture	150	145	554	1646	9	6	12	73
Fallow	57	166	366	1003	5	10	14	72
Intensive	249	115	699	1992	12	4	14	69
Minimum	98	84	458	1276	9	5	14	72
No tillage	112	104	429	1454	10	6	12	72
I.s.d.	40.8	44	124	242	2.4	0.86	2.5	3.6

Table 5. Solubility in hot water of C in particle-size fractions of soils under different treatments and sampling depths.

Depth (cm)	Fraction	C solubility in hot water (mg HWC g ⁻¹ fraction C)				
		Pasture	Fallow	Intensive tillage	Minimum tillage	No tillage
0–7.5	Clay	55	39	39	40	42
	Fine silt	47	38	42	45	47
	Sand (POM)	72	37	54	56	54
I.s.d.		10.4	12.7	6.2	8.8	10.8
7.5–15	Clay	44	36	37	37	37
	Fine silt	45	34	41	43	40
	Sand (POM)	59	21	55	39	46
I.s.d.		9.0	8.3	3.5	6.4	14.9
15–25	Clay	33	28	36	28	31
	Fine silt	34	35	38	31	31
	Sand (POM)	40	20	56	30	44
I.s.d.		10.1	10.5	6.2	11.0	17.8

HWC, hot water extractable C; POM, particulate organic matter.

each size fraction, there was a linear relation between total fraction C and C extracted in hot water (Fig. 2) and, as with the relationship between total soil C and soil HWC, there was a significant intercept, suggesting that part of the

organic matter in the size fractions did not release C to hot water. The amount of ‘insoluble C’ (i.e. intercept value on the x-axis; Fig. 2) was greatest in the clay fraction (7.2 ± 0.5 g kg⁻¹ soil; $P < 0.001$). Intercept values (g kg⁻¹ soil) for

the fine silt, coarse silt and POM were 0.8 ± 0.1 ($P < 0.001$) 0.18 ± 0.07 ($P = 0.03$) and 0.7 ± 0.1 ($P < 0.001$), respectively. The summed intercept values of the size fractions (9 g C kg^{-1} soil) corresponded closely with the whole soil value (9.6 g C kg^{-1} soil). When HWC was plotted against 'total labile C' (here defined as total C minus the 'insoluble C'), the relationship was generally similar for whole soil and particle-size fractions (Fig. 3). Averaged across soils and particle-size fractions, hot water extracted 85 mg C g^{-1} of total labile C. The solubility of fine silt C in hot water was somewhat low compared with other fractions (58 mg HWC g^{-1} of total labile C vs $76\text{--}77 \text{ mg HWC g}^{-1}$ of total labile C in the clay and sand fractions).

Discussion

Physical fractionation and chemical extraction procedures share a common goal of isolating and quantifying labile SOM. In this context, an understanding of how different soil fractions release organic C to specific extractants (hot water in this case) may contribute to better appreciation of the connection between physical and chemical partitioning approaches. We exploited the wide range of total C and HWC found in soils from a previous trial site (resulting from the diverse treatments that had been imposed; different depths of sampling) to examine the C-release characteristics of particle-size fractions in the absence of possible confounding factors (e.g. due to mineralogical or textural differences).

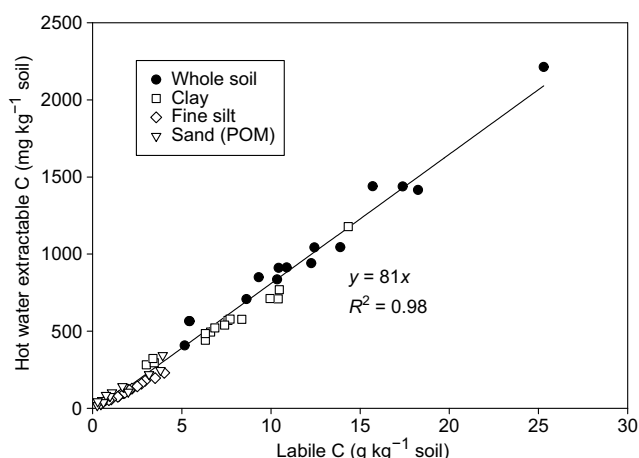


Fig. 3. Relationship between hot water extractable C and total labile C (total C minus 'water insoluble' C) in whole soil and soil particle-size fractions (clay, fine silt and sand). The 'water insoluble' C was estimated as the x-axis intercept value in Fig. 2. Data represent different sampling depths (0–7.5, 7.5–15 and 15–25 cm) under permanent pasture, bare fallow and arable cropping using intensive tillage, minimum tillage and no-tillage. POM, particulate organic matter.

Change in land use from long-term grazed pasture to arable cropping resulted in an average decrease in total C stocks to 25 cm of 14% (11 t ha^{-1}), with a greater decline (23%) in C stock in soil that had been chemically fallowed for 13 years. Loss of C from continuous fallow soil, which was not physically disturbed during the trial, can be attributed to the absence of C inputs combined with favourable soil moisture conditions for SOM mineralisation (due to lower evapotranspiration than in treatments with plant cover). The decline in C under arable cropping can also be ascribed to reduced C inputs, particularly root C inputs, which contribute relatively more to soil C maintenance than above-ground C inputs (Kätterer *et al.* 2011). Lack of effect of tillage intensity on C stocks is consistent with numerous reports that, while the distribution of C in the profile may be affected by tillage method, total soil C storage may not be enhanced by adoption of low-disturbance tillage methods (Wiesmeier *et al.* 2019). In the present trial, crop production (and, by extension, C inputs in crop residues) did not differ consistently between tillage treatments (Beare *et al.* 2015).

The HWC was more sensitive to the imposed treatments than was total soil C. Thus, in addition to the decline in total C stocks under the C-depletive treatments (arable cropping or bare fallow), the solubility of the organic C remaining in these treatments was significantly lower than in the reference system (pasture soil), i.e. C solubility decreased from 55 mg g^{-1} of total C in pasture soil to 45 and 37 mg g^{-1} C, respectively, in cropped soil and bare fallow. Previous research at this site showed a close linear relationship between HWC and C mineralised (in 14 weeks) from these whole soils under optimal conditions of temperature and soil moisture (Curtin *et al.* 2020). Linear regression analysis in this study suggested that part of the SOM did not release C to hot water. This 'insoluble C' (estimated at 9.6 g C kg^{-1} soil from the relationship between HWC and total soil C), which may comprise soil organic C that is biologically recalcitrant (Curtin *et al.* 2020), represented between about one-quarter (pasture surface soil; high C soil) and two-thirds (15–25 cm layer of fallow and low-disturbance tillage soils; low C soils) of total soil C. The greater sensitivity of HWC (cf. total C) to the experimental treatments can be attributed to the specificity/selectivity of hot water for the labile portion of SOM. This is consistent with previous work in New Zealand showing that the impact of land use on HWC was greater than that on total soil C (Ghani *et al.* 2003).

The size fractionation results showed that, while there was a relatively large decline in POM-C in the bare fallow treatment, this fraction accounted for only about one-quarter of the C lost ($\sim 19 \text{ t C ha}^{-1}$) from this treatment during the 13-year trial period. Although SOM residing in the clay fraction was more stable than POM, it was a much larger C pool and accounted for much (>50%) of the C lost from whole soil. Collectively, the silt fractions accounted

for one-quarter of the C lost from fallow soil, with most of this originating from the fine-sized silt. These results are consistent with those of Meyer *et al.* (2017), who found that 64% of the soil C lost during an 11-year bare fallow experiment in Germany originated from the non-POM fraction (<20 μm fraction).

The C lost as a result of land-use change from pasture to arable cropping also appeared to originate primarily from the clay fraction, with surprisingly little loss of POM-C. In an effort to ameliorate soil physical degradation under arable cropping, 'restorative' crops (i.e. ryegrass seed crops) were incorporated into the rotation during the 2008–09 period (18 months), and in the years (2012–13) immediately prior to our soil sampling. Inputs of organic matter, particularly root biomass, from these restorative crops can be substantial (Francis *et al.* 1999) and may have helped replenish the stock of POM. However, the relatively short period under ryegrass was insufficient to restore the large pool of C associated with the clay and silt fractions. Our results are in keeping with the finding of McNally *et al.* (2018) that the majority of the C lost under continuous arable cropping in New Zealand was derived from the <50 μm fraction.

All of the particle-size fractions released C to hot water, with the clay fraction being the predominant source (59–77% of C extracted from the size fractions originated from the clay fraction). In each fraction, as in whole soil, there was a linear relationship between total fraction C and HWC. The form of the relationship suggested that organic matter in the size fractions was a composite of stable (C insoluble in hot water) and labile components, with the labile component releasing C to hot water at an average rate of $\sim 80 \text{ mg g}^{-1}$ of total labile C. The stable material was concentrated in the clay fraction ($7 \pm 0.5 \text{ g C kg}^{-1}$ soil), but there was evidence that even the POM fraction included a stable component ($0.8 \pm 0.1 \text{ g C kg}^{-1}$ soil). This helps explain the observation that, even after more than 50 years with very minimal inputs of plant C, some POM-C was recovered from the Long Term Bare Fallow plots at Rothamsted, UK (Curtin *et al.* 2017a).

Our results indicate that the organic matter in the other physical fractions (clay and silt) is also heterogeneous, with the proportions of labile and stable organic matter in each fraction differing between treatments and sampling depths. For example, 67% of clay-C in the top 7.5 cm of pasture soil was in the labile category compared with only 30% in fallow soil at the 15–25 cm depth. The relationship between HWC and total labile C was reasonably similar across all fractions (Fig. 3), albeit with organic matter in the fine silt fraction being slightly less soluble in hot water. We do not have a cogent explanation for the latter observation.

The information obtained from our study is invaluable to understanding the link between the C stored in particle-size fractions and HWC. Similar work at other sites with diverse

soil types will yield additional information to advance our understanding of the inter-relationships between the solid-phase C and water-extractable C.

Our physical fractionation procedure, which involved isolation of four size fractions, provided more detailed information on the particle-size distribution of C than would be possible with the recently proposed protocol to partition SOM into just two components: POM and MAOM (<50 μm fraction) (Cotrufo *et al.* 2019; Lavallee *et al.* 2020). Dividing the MAOM into sub-fractions, as done in the present study, requires considerable time and labour, and it is debateable whether the additional information justifies the effort. As discussed, the proportions of labile and stable organic matter in the <50 μm fraction can vary depending on land use and other factors (e.g. sampling depth) and, so, a measure of the quantity of MAOM, in itself, is unlikely to be a reliable predictor of the response of SOM to management or environmental perturbations. Information on MAOM quantity needs to be supplemented with data on its bioavailability. Assays using chemical oxidising agents have been proposed to enable the stable organic matter component (resistant to oxidation) to be isolated and a combined physical–chemical fractionation could, potentially, be an effective way to partition SOM into fractions with distinct turnover rates (Poeplau *et al.* 2018). However, as yet, a chemical oxidation procedure capable of distinguishing labile from stable organic matter has not been identified (Lutfalla *et al.* 2014). The results of this study suggest that HWC may offer a relatively simple and practical method to distinguish the recalcitrant and labile pools of C associated with the physically isolated POM and MAOM fractions.

Summary and conclusions

A field trial with a diverse set of treatments (pasture to arable conversion, ex-pasture soil maintained fallow for 13 years and arable cropping with contrasting tillage treatments) provided a template to evaluate two widely used approaches to characterise SOM: particle-size fractionation and hot water extraction. As expected, POM-C was the most responsive of the size fractions to imposition of the fallow treatment (POM-C stock declined by 47%); however, >50% of the C lost during the 13-year fallow period was derived from the clay fraction (the largest and most stable C fraction). A large proportion of the C lost under arable cropping was also derived from the clay fraction. Our results highlight a significant weakness in the particle-size approach to SOM fractionation, i.e. labile SOM is spread across all size fractions, with the relative proportions of labile and stable organic matter in each size fraction varying depending on land use, sampling depth and, presumably, other factors. Ideally, measurements of the quantity of organic matter in

size fractions should be complemented by data on its bioavailability; however, a validated procedure that is specific for the labile C component is not available.

All size fractions contributed to soil HWC, with the clay fraction being the dominant source. Soil HWC was more responsive to the experimental treatments than was total soil C, adding to evidence that the hot water method measures a labile component of the SOM. We established a relationship between total labile C (defined as total C minus C that is insoluble in hot water) and HWC that appeared to be consistent across size fractions and whole soils. Although the hot water extraction method has the advantage (vis-a-vis size fractionation) that it is selective for labile C, hot water is a weak extractant that recovers a relatively small proportion of the labile C; for our soil (~80 mg C extracted per g of total labile C). The relationship observed here between HWC and labile C might allow estimation of labile C from a HWC measurement; however, further research is needed to verify whether this relationship is soil-specific or can be applied generally. Future work should include a focus on the soil and environmental factors that may influence the relationship between labile (bioavailable) soil C and HWC, and their association with soil physical fractions.

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Data availability. The data that support our findings are available from the corresponding author upon reasonable request.

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Author affiliation

^AThe New Zealand Institute for Plant and Food Research Limited, Private Bag 4704, Christchurch, New Zealand.