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Storage of soil carbon as particulate and mineral associated organic matter in irrigated woody perennial crops

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ABSTRACT

Agricultural practices such as annual crop production, land use change and grazing on marginal lands lead to a loss of soil carbon (C) stock. But soil C losses are not universal in agricultural systems and modest soil C gains can occur when constraints such as a lack of water are removed. To characterize this we used a meta-analysis of published data focused on semi-arid regions, where irrigation is required for crop production. We showed that soil C stocks declined under cereals, cotton, maize and non-woody horticultural crops when compared to native unirrigated adjacent grassland or shrubland. By contrast, cultivation of irrigated, woody perennial crops generally leads to an accumulation of soil C. Identifying the mechanisms by which C is retained in the soils beneath woody perennial crops, and any limits to C accumulation, was the main goal of this study. A mechanistic understanding of soil organic C content accumulation, upon land use change, can be gained by dividing soil into particulate organic matter (POM) and mineral associated organic matter (MAOM). Here, we analyzed the C and natural abundance ¹³C concentrations in POM and MAOM fractions in soils from eight apple orchards and eight vineyards irrigated using a dripline, and eight apple and eight cherry orchards irrigated with micro-spray. Samples were also taken from eight native grassland areas adjacent to the agricultural sites for comparison. Several decades of woody crop production doubled the average soil C concentration in comparison to the native sites, from to 10.1 ± 1.48 g C kg⁻¹ to 20.1 ± 0.96 g C kg⁻¹ over a depth of 0–15 cm. Most of the C was associated with POM, which increased in concentration from 7.9 ± 1.19 g C kg⁻¹ to 14.2 ± 0.79 g C kg⁻¹ in 0–15 cm soils, an increase of 80%. This was crop dependent, being highest in the cherry orchards and lowest in the vineyard soils. Although holding less C, the MAOM concentration increased by 166%, changing from 2.22 \pm 0.33 to 5.91 \pm 0.62 g C kg⁻¹; no differences existed between crops but the MAOM C concentration appeared to be constrained to a maximum value of ~ 12 g C kg⁻¹. MAOM and POM had markedly different δ^{13} C values: MAOM was more enriched, indicative of greater microbial processing, whereas POM had a lower $\delta^{13}C$ value consistent with the dominant standing vegetation. δ^{13} C values were more depleted in both fractions at the agricultural sites compared to the native sites, indicating the accumulation of greater amounts of less processed C at the agricultural sites, due to higher C inputs. We conclude the soils in this region respond to irrigated perennial woody crop production by retaining C within both POM and MAOM fractions. Accumulation of C as MAOM is constrained by the minerology of the soils in this region, but is unconstrained for POM which dominates the soil C content, and is potentially vulnerable to changes in management practices and land use.

1. Introduction

Soil organic matter is the largest terrestrial carbon (C) pool and contains three times the amount of C as either the atmosphere or in terrestrial vegetation (Schmidt et al., 2011). It has been the subject of

intense research for the last two decades (Schmidt et al., 2011; Sokol et al., 2019); much of this work has focused on the protection of soil organic matter, and building an understanding of what controls the C content of soil and its response to climate change. Over ten thousand years, agriculture has reduced global soil C content by 116 Gt, mainly

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through the conversion of native ecosystems into agricultural land, and the degradation of grazing land (Sanderman et al., 2017); this is a C loss equivalent to a decade of current industrial CO₂ emissions (Amundson and Biardeau, 2018). Management practices that promote the accumulation of soil organic C (SOC) are attractive, because they not only address this soil C debt, but offer the potential to contribute to the longlasting mitigation of rising atmospheric CO₂ levels, and provide a range of other co-benefits, such as more active nutrient cycling and higher crop productivity (Schmidt et al., 2011; Wiesmeier et al., 2019).

Soil organic C falls broadly into two distinct pools: particulate organic matter (POM) and mineral-associated organic matter (MAOM) (Cotrufo et al., 2019; Haddix et al., 2020; Lavallee et al., 2020). Particulate organic matter is mainly composed of fragmented, relatively undecomposed plant litter, is vulnerable to disturbance, and has a more rapid turnover time than MAOM (Lavallee et al., 2020; Poeplau et al., 2018). Mineral-associated organic matter is thought to comprise small molecular weight compounds resulting from the microbial processing and depolymerization of POM (Cotrufo et al., 2019). The combination of strong physico-chemical sorption and spatial separation from microbial decay through protective aggregates means MAOM turns over much more slowly than POM and can remain in soils for long periods of time (Mikutta et al., 2019; Sokol et al., 2019). From a climate change mitigation perspective, increasing soil C content of the MAOM fraction is more desirable than increasing the POM fraction, since this represents a more stable, long-term C reservoir in soils. Nevertheless, as elegantly discussed by Janzen (2006), a careful balance must be struck between locking away C and allowing SOC turnover to release nutrients and fuel plant productivity.

Perennial woody crops such as grapes and orchard fruits have a number of characteristics that, unlike annual crops, make them attractive for long-term C storage under appropriate management regimes. Perennial woody crops have long life-spans, their cultivation involves very infrequent or no soil tillage, and herbaceous vegetation in the alleys between the tree or vines rows can contribute to soil C inputs (Midwood et al., 2020; Scandellari et al., 2016; Tezza et al., 2019). By fractionating the soil beneath woody perennial crops into MAOM and POM, a more mechanistic understanding of SOC cycling processes can be achieved. For example, the microbial efficiency matrix stabilization framework, developed by Cotrufo et al. (2013), provides a mechanistic link between litter inputs and these soil fractions. Plant litter inputs rich in complex forms of C, such as lignin, have a high energy cost associated with their breakdown and low so-called microbial use efficiency. The microbial efficiency matrix stabilization concept suggests this restricts C flow to the MAOM fraction and tends to increase POM content (Cotrufo et al., 2013; Samson et al., 2020); woody pruning debris in orchard and vineyards might fall into this category. By contrast, soil C inputs from herbaceous plants growing in the alley rows, such as root exudates or fine root turnover, would foster MAOM formation since these C sources are readily decomposed by the soil microbial community (Cotrufo et al., 2013). MAOM formation may not be entirely dependent on litter quality, however, and it has been argued that direct sorption of microbial and plant-derived compounds onto soil mineral surfaces may also be an important mechanism, especially in free-draining soils (Mikutta et al., 2019; Sokol et al., 2019). Irrespective of precisely how MAOM is formed, by analyzing the POM and MAOM fractions important insights into the underlying mechanisms driving overall SOC accumulation, or decline can be obtained.

Tillage is another example where a more mechanistic understanding of overall soil C changes can be achieved by considering the POM and MAOM content. Reduced tillage in cereal and grape production has been shown to increase the soil C content especially at the surface; this increase has been attributed predominantly to increases in POM content and, to a lesser extent, in MAOM content (Belmonte et al., 2018; Blanco-Moure et al., 2013; Samson et al., 2020). Increases in POM occur when this fraction is protected from microbial decomposition by aggregation, and specific soil environmental conditions exist, such as low oxygen levels, water saturation and freezing temperatures (Trumbore, 2009). Tillage disrupts protective aggregates, increases soil oxygen levels, and changes soil drainage, thereby increasing POM decomposition rates; by contrast, MAOM decomposition rates are much less sensitive to these changes (Lavallee et al., 2020). The overall impact of no till on agricultural soils is contentious though, and in many cases apparent increases in soil C in the surface soil are off-set by re-distribution of C deeper in the soil profile, meaning that the overall soil C content may not change (Mary et al., 2020; Powlson et al., 2014). Crop yields and residues returns to the soil may also be influenced by tillage (Mary et al., 2020). These observations emphasize the importance of sampling below the top 0–20 cm or plough layer to fully assess the impact of management practices on overall soil C concentration and stock levels.

The natural abundance of the stable isotope 13 C in POM and MAOM is different; δ^{13} C in POM is typically more similar to current plant litter inputs than the MAOM, which is enriched in 13 C as a result of microbial processing (Poeplau et al., 2017). By combining stable isotope analysis with the separation of POM and MAOM, the opportunity exists to track the fate of new litter C, with its characteristically low 13 C content, into these fractions. This can provide new insights into the response of the overall soil C content to differing C input rates. The results of a recent regional study of over 80 commercially managed vineyards and orchards in south-central British Columbia, Canada showed that soil C content responds rapidly to the crop being grown and its associated management practices (Midwood et al., 2020). We speculated that the observed differences among crops (apples and cherry orchards, vine-yards) are primarily driven by differences in plant C inputs to the soil, including plant litter, root exudation and turnover.

The objectives of this study were to identifying the mechanisms by which C is retained in the soils beneath woody perennial crops, and any limits to this accumulation. To set the context, we first conducted a metaanalysis of previously published peer-reviewed articles to assess the impact of irrigated agriculture on soil C stock changes in semi-arid regions, measured by comparison to adjacent native grassland soils. Then, we took a sub-set of soil samples from our previous study (Midwood et al., 2020), as well as soils from adjacent native grassland sites, split them into POM and MAOM fractions, and analyzed the total organic C and ¹³C content. We used the ¹³C content of the C in the POM and MAOM to determine how incorporation of recent C inputs into these two fractions differed between native and agricultural soils, and among crops with contrasting C input rates. The formation of MAOM is critically linked to the availability of reactive mineral surfaces capable of stabilizing organic matter (Sokol et al., 2019); so we used textural analysis of the soils to compare clay contents with MAOM contents in order to better characterize the capacity of these soils to retain C in the form of MAOM. Our work set out to address three hypotheses: 1) Most C in the soils of this region accumulates in POM fraction, and this is dependent on crop type; 2) due to limited mineral weathering, storage of C as MAOM is constrained; but 3) clay and MAOM content are correlated. The ultimate goal of our work was to build a better understanding of the C storage potential of the soils across this region and the scope which exists, through appropriate management practices, to increase soil C content over the long term.

2. Methods

2.1. Meta-analysis

A systemic literature search was conducted to identify peer-reviewed publications that reported SOC data from irrigated agricultural fields and adjacent native sites in semi-arid regions. We used search criteria with the following keywords: irrigation; soil organic carbon; agriculture; aridity; climate; soil texture; soil depth and change over time, to select peer reviewed research papers from the Web of Science and Google Scholar. After the initial search, all the papers were systematically assessed to determine their suitability for inclusion in the *meta*-analysis using a set of pre-defined criteria. Only data from field studies were included (i.e. no greenhouse or laboratory studies). Also excluded were i) data from experiments conducted in rice paddies or forests, ii) papers that provided insufficient detail about study design, iii) papers that included only one growing season, and iv) papers that used irrigation water sources other than fresh water. The final literature search was completed on January 14, 2019 and included 15 studies published between 1974 and 2017, (see Section 1.2 in the Supplemental Information). Data on SOC concentrations or contents, by soil depth, in irrigated and native sites, as well as information on crop type and tillage method were extracted from each selected publication and entered into a database. Soil organic carbon data collected from 'native' sites were considered control data and SOC data collected in the same year from adjacent, irrigated agricultural sites were considered 'treatment' data. If multiple years of SOC data were reported, data were taken from the final year of the study only, to avoid pseudo-replication. When data were presented in figures, rather than tables, values were estimated using WebPlotDigitizer (https://apps.automeris.io/wpd/). Standard deviation (SD), and number of replicates (n) were also recorded; where necessary, standard error was converted to SD where SD = SE $\times \sqrt{n}$. Soil depth measurements varied between studies. In order to standardize comparisons by depth increment, values were fitted to a specific depth category by first finding the median of the reported depth increment, and then determining the depth category (0-15, 15-30, >30 cm) into which the median value fell. Where studies reported more than one SOC measurement in one depth category, a single value was calculated, using a weighted average.

Most papers reported SOC data as either C concentration (i.e., SOC kg^{-1} dry soil) or C stock (Mg SOC ha^{-1}). Given that changes in SOC stock most accurately reflect changes in soil carbon storage, SOC concentration data were converted to SOC stocks, when necessary, using the following equation:

SOC stock (Mg C ha^{-1}) = SOCconc.*BD*t*0.1

where SOCconc is soil organic carbon concentration in $g kg^{-1}$, BD is bulk density in g cm^{-3} , t is the thickness of the depth increment (cm), and 0.1 is the conversion factor for Mg ha^{-1} . Despite its importance for determination of soil properties and SOC stock, bulk density (BD) was not reported in all studies. To calculate the missing bulk density values, we developed a novel pedotransfer function, which applied a random forest algorithm to each soil depth category in the dataset, including all available predictor variables. Random forest works by combining a large number of regression trees, trained using bootstrap aggregation, to build a robust predictive model that is resistant to noise in the data. The R code for this model is available at https://github.com/dsemde/Emd e-et-al.2021-public. We did not, however, use this approach to estimate bulk density values for the Holland et al. (2016) paper because this study was conducted in the Okanagan Valley on similar site types; as such, we employed average bulk density values measured in our study to convert the SOCconc data reported in Holland et al. (2016) to SOC stock.

The difference in SOC stock due to irrigated agriculture was calculated as:

% difference SOC stock = (agricultural SOC stock

- natural SOC stock)/natural SOC stock*100

Analyses were conducted by soil sample depth category because previous *meta*-analyses have shown that sample depth is a strong predictor of management-caused changes in soil C storage (Bai et al., 2019; Du et al., 2017). 95% confidence intervals were determined for data from each depth interval. All analyses associated with the *meta*-analysis were carried out in R version 1.2.1578 (R Core Team, 2019) using the '*metafor*' package (Viechtbauer, 2010).

2.2. Soils and sampling sites

Soil samples were collected in 2018 from 40 separate locations along the Okanagan Valley, a semi-arid region approximately 200 km in length running in a north-south orientation in the southern interior of British Columbia, Canada. The sites and soil samples used in this study were taken from a larger regional study, which has been described in Midwood et al. (2020). Briefly, the soil samples used were collected from five different crop systems: 1) apple orchards and 2) vineyards irrigated by drip-line systems, 3) apple orchards and 4) cherry orchards irrigated by micro-spray systems, and 5) non-cultivated, native vegetation sites spread along the length of the valley. The term 'crop system' refers to the combination of crop and irrigation type. These crop systems are typical of this region of Canada. We selected eight replicates of each crop system plus native sites, providing soil samples from a total of 40 separate sites (8 of each crop system plus 8 native sites); the sites were selected to have a broad geographic spread and included soil types typical of those used for perennial agriculture in this region (Table S2). All of the orchard and vineyard sites were commercially managed and, as such, they were subjected to a broad range of real-life management practices including irrigation, fertilizer applications, pruning and weed/pest control. The native sites were located close to a number of our agricultural sites and, thus, shared the same soil classification, elevation, climatic conditions and aspect; typically, they consisted of natural grassland or shrubland over the fence line or in awkward parcels of land inaccessible to farm machinery.

At each site, soil samples were collected using a 5-cm diameter auger at three depths: 0-15 cm, 15-30 cm and 30-60 cm. These depths were selected to ensure full characterization of any C concentration changes beyond the surface layer (Powlson et al., 2014). Each 'site sample' was a composite of between 10 and 20 individual samples located randomly over an area of approximately 0.25 to 0.50 ha at each site. Soils were initially sieved through an 8-mm screen, then air dried and sieved through a 2-mm screen, stones and large roots fragments were removed at this stage, but no soil discarded. The soils were analyzed for total organic and inorganic C, total N, pH, exchangeable Ca, Mg, K and Na, and texture; the details of the methods used are provided in the Supplementary Material (Section 1.1 and Table S3). Carbon stocks were estimated using the organic C content and bulk density measurements taken at two depths (between 0 and 15 cm and 15-30 cm) from six locations at the cropped sites and three at the native sites, using the method described by Midwood et al. (2020). Briefly, a pre-weighed copper ring (5x5 cm, height \times diameter) was hammered into the soil starting at a depth of 5 cm until completely flush with the surface at this depth, the ring was then excavated, and the top and bottom openings covered with foil and taped in position to retained the un-disturbed soil contents. This procedure was then repeated at 20 cm below the soil surface in the same location. It was not possible to measure bulk density below this depth because stones prevented the rings being hammered into place. The soil and collars were returned to the laboratory and dried at 60 °C to a constant weight; any coarse fragments (stones or large root fractions) were then quantified and removed while sieving the soil to 2 mm. Soil C stock was calculated from the bulk density measurements using the approach described by Hobley et al., (2018).

2.3. Soil fractionation

All the soil samples were fractionated based on the method described as 'Par-Den 5' in Poeplau et al. (2018); this method combines both flotation and wet sieving and was evaluated by Poeplau et al., to produce a series of mechanistically distinct fractions. Briefly, 50 g of air-dried 2 mm-sieved soil were dispersed in 150 ml of deionized water with seven 5-mm diameter glass beads overnight (16 h) on a shaker at 200 rpm. The soil suspension was then sieved through 200 μ m and 50 μ m screens. The fraction retained on the 200 μ m screen was re-suspended in deionized water and any floating material collected separately from the > 200 μ m dense fraction. The Par-Den 5 method describes the collection of a second light floating fraction, from the material held on the 50 µm screen, a 'Light 200 to 50 µm' fraction. However, the soils in our study did not have any light fine material falling into this size category and so this fraction was not collected. All the fractions (2000-200 µm light mainly coarse plant debris; 2000-200 µm dense fraction - coarse sand; 200–50 μ m dense fraction - fine sand; and < 50 μ m fraction - silt and clay) were oven dried at 50 °C and hand-ground to the consistency of flour prior to isotope analysis. On average we found the accumulated weight of the four separated fractions accounted for 94.3% of the initial soil weight (ranging from 90.8 to 96.8%), illustrating good overall recovery and indicating that the loss of any dissolved organic C was small. After an initial analysis of the fractions, a decision was taken to simplify our methodology and focus on just the two critical fractions: MAOM and POM, following the approach taken by Cotrufo et al., (2019). We designated the $< 50 \ \mu m$ (silt and clay) fraction as MAOM. The data obtained from the three remaining fractions were combined to generate a single POM fraction; the overall ¹³C value for the POM was calculated using mass balance approach. The 2000-200 µm light material, although C-rich with an average C concentration of 35%, only constituted 0.1% of the mass recovered and was highly variable. The bulk of POM (approximately 97% of the POM C) was in the two sand fractions; the 2000-200 µm dense fraction (coarse sand) contributed on average 67% of the mass recovered and the 200–50 μ m dense fraction (fine sand) contributed 33% of the mass recovered. The average C concentration in both fractions was identical at 1.3%; this, together with a general lack of aggregation in the sandy soils of this region, suggests the POM in these fractions was not functionally different and so combining all these fractions into a single POM sample was justified. The separation of the POM from the MAOM fraction was not perfect, our textural analysis results indicated that on average the sand content of our samples was 49%, while mass recoveries from our separations, adjusted for average sample loss, indicated the sand content was higher, at 56%. The difference was greatest in those soils with a high silt content, and it may be that coarse silt fractions approaching 50 µm in size were retained on the sieve with the fine sand fraction during our aggregate separation procedure. Organic matter associated with coarse silt fractions is thought to have similar characteristics to POM and be relatively labile (Curtin et al., 2019) so the overall impact on our results would have been relatively small.

2.4. Soil carbon analysis

Organic C and δ^{13} C of the intact soil and associated fractions were measured, after treatment with 2 N HCl to remove any inorganic C, using an IsoPrime continuous-flow mass spectrometer (GV Instruments, Manchester, UK) linked to a EuroVector elemental analyser (Milan, Italy). A two-point linear normalization procedure, using two in-house working standards, was used to standardize the isotopic analysis (Skrzypek, 2013). The working standards were calibrated to V-PDB through the National Institute of Standards and Technology reference materials sucrose RM 8542 and polyethylene RM 8540.

2.5. Statistical analysis

For the field study two-way ANOVA was used to analyze overall differences in the total C content, δ^{13} C values and relative proportions of the POM and MAOM between agricultural and native sites, and among the three depths. Differences in the total C and δ^{13} C content of POM and MAOM between the native sites and crop systems over three depths were also assessed using a two-way ANOVA. To test whether clay content was a covariate in our data, individual scatter plots for the four cropping systems and the native sites for each of the variables tested were created. No systematic variation was observed so clay content was not added as a covariate to the statistical tests. All data were transformed as required to meet the requirements of normality prior to ANOVA. The same approach

was used to compare the other soil parameters measured (e.g., pH, exchangeable Ca; Table S3). *Post-hoc* pairwise comparisons were made using Tukey's Honestly Significant Difference analysis. Analysis of covariance was used to compare lines of best fit, applied to changes in MAOM C content with soil clay content to determine if either the gradient or intercepts were different. All analyses were undertaken using the R statistical package, version 1.2.1578 (R Core Team, 2019). All reported values with errors, are means \pm one standard error.

3. Results

3.1. Meta-Analysis

Our literature search identified 15 peer-reviewed studies conducted in semi-arid regions in which soil C stocks in irrigated agricultural sites and adjacent native sites were compared (Table S1). These studies were published between 1974 and 2017, and originated in Canada, Brazil, the United States, Australia, Iran and China. In all of these studies, the native sites were characterised as either grassland or brushland. Fourteen of the studies reported data from annual cropping systems, and these were used for the *meta*-analysis. One of the fifteen studies contained data from vineyard soils, a perennial cropping system; because this was very different from the remaining 14 studies it was considered separately. It should be noted that three of the 14 studies used in the *meta*-analysis were conducted in cotton, a woody perennial; however, cotton is typically managed as an annual crop, i.e., grown for one year and then chemically defoliated (and killed) prior to harvest.

According to our *meta*-analysis, soil C stocks at the 0–15 cm depth were 10.3% lower, on average, in irrigated annual production systems than in native sites. Soil C stocks at the 15–30 cm depth (and below) were similar in irrigated annual production systems and adjacent native sites (Fig. 1). Overall, soil C stocks in irrigated annual production systems changed significantly and were 5.45% lower than in native sites.

One study identified during our literature search reported data from native sites and perennial cropping systems (vineyards) in the Okanagan



Fig. 1. Changes in soil C stock (%) in irrigated agricultural soils, relative to adjacent native soils. 'Overall' values are the average values from all soil depths. Error bars are \pm 95% confidence intervals; numbers by each data point indicate the number of observations (study sites) used to calculate mean values. Vertical dashed line indicates no difference in soil carbon between native and irrigated sites; when error bars do not cross the vertical dashed line, soil C stocks in native sites are considered significantly different from C stocks in agricultural sites.

Valley (Holland et al., 2016). Because this study originated in the same region as our study and was the only relevant study conducted in longlived woody perennial crops, we did not include these data in Fig. 1. On average, soil C stocks at the 0–30 cm depth were 29.5% greater in vineyards than in native sites, but treatment effects were not statistically significant.

3.2. Soil C concentrations

In our field study, organic C concentrations in both native and agricultural soils were greatest in the 0–15 cm depth interval (Fig. 2a,d). Concentrations of both POM C, which constituted ~ 75% of the total C content, and MAOM C declined with depth (p < 0.001). Cultivation doubled the average organic C concentration at 0–15 cm depth interval, from 10.1 ± 1.48 g C kg⁻¹ dry soil (sum of POM and MAOM; n = 8) in the native soils (Fig. 2a), to an average of 20.1 ± 0.96 g C kg⁻¹ (n = 32) across all crop systems in the agricultural soils (Fig. 2d). This change was a result of an 80% increase in the POM C concentration (P < 0.001) and a 166% increase in the MAOM C concentration with respect to the native soils (P < 0.001). The relative proportion of C present as POM relative to the MAOM decreased slightly with depth (POM p = 0.0142, MAOM p = 0.0081), but did not differ between the native and agricultural sites (Fig. 2b,e, Table S4).

The effect of irrigated woody perennial fruit cultivation on POM and MAOM C concentrations relative to native soils was more pronounced in surface soils and declined with depth (POM p < 0.001; MAOM p = 0.0057) (Fig. 3). The POM C (p = 0.0286), but not the MAOM C, differed among the four cropping systems. Production of cherries caused the largest increase in POM in the surface soils (0–15 cm) 9.15 \pm 1.47 g C kg⁻¹, while production of grapes increased POM C by only 2.85 \pm 0.80 g C kg⁻¹ (Fig. 3, Table S5). The effects of all other cropping systems were intermediate. Neither POM nor MAOM C concentrations showed significant interaction between cropping system and depth.

Calculation of the overall soil C stocks, from a combination of the POM and MAOM C contents, showed that at the native sites, the average soil C stock was 22.5 Mg C ha⁻¹ at 0–15 cm, and 38.2 Mg C ha⁻¹ at the agricultural sites, an increase of 69.5%. At a depth of 15–30 cm, the average C stock at the native sites was 13.5 Mg C ha⁻¹ and 23.1 Mg C ha⁻¹ at the agricultural sites, an increase of 71.3%. On average, over both depths, soil C stocks were 70% greater in irrigated woody perennial crop systems compared to native sites; all of the C stock increases were significantly different from the native sites.

3.3. Soil natural abundance $\delta^{13}C$ values

Natural abundance δ^{13} C analysis was used to trace the incorporation of photosynthetically-fixed C into the SOC. Isotope discrimination during photosynthesis means that recent C inputs are typically depleted in ¹³C relative to the existing soil C, and microbial processing tends to increase the ¹³C content of the soil C (Ehleringer et al., 2000). In both native and agricultural sites, the POM and MAOM were depleted in ¹³C in surface soils relative to deeper soils (p < 0.001, Fig. 2c,f.). Across both site types and all depths, the δ^{13} C value of POM was 0.8 ± 0.22‰ (n = 24) more depleted than the MAOM. The δ^{13} C values of both POM and MAOM in soils from agricultural sites were depleted relative to soils from the native sites (POM ¹³C p = 0.0097; MAOM ¹³C p = 0.0174) (Fig. 2c,f. Table S4).

The difference in POM $\delta^{13}C$ values between agricultural and native soils varied with depth (p < 0.001) (Fig. 3, Table S5) but not by cropping system. The $\delta^{13}C$ values of POM at the agricultural sites differed from the native soils in a similar pattern across all cropping systems, being most depleted at the 0–15 cm and 30–60 cm intervals. By contrast, the difference in MAOM $\delta^{13}C$ values between agricultural and native soils varied by cropping system (p = 0.0064); MAOM in cherry orchard soils was more ^{13}C depleted with respect to the native soils than MAOM in micro-spray irrigated apple orchard soils, whilst MAOM in vineyard and drip irrigated apple orchard soils were intermediate. The difference in MAOM $\delta^{13}C$ values between agricultural and native soils did not vary with soil depth.



Fig. 2. Organic C concentrations, relative mass proportions and δ^{13} C values of POM and MAOM fractions by soil depth at the native (a, b, c) and agricultural (d, e, f) sites. Open circles/open bars are POM fractions and filled triangles/fill bars are MAOM fractions. Errors bars are ± 1 SE, n = 8 for the native sites and n = 32 for the agricultural sites. Two-way ANOVA summary comparing the two sites is shown below the figure, with a complete ANOVA table shown in Table S4.



^{*}Letters on graph illustrate which crop system led to these significant differences, POM C to left of crop title and to the right of the crop title, MAOM ¹³C.

Fig. 3. Difference in fraction C and δ^{13} C values between the native and individual agricultural site types (drip-irrigated vineyards, microspray and drip-irrigated apple orchards, microspray-irrigated cherry orchards), where open circles are POM and filled triangles are MAOM. Differences were calculated from the means of the agricultural – native sites, such that values > 1 in 'Fraction C' indicate an increase in agricultural sites and values < 1 in 'Fraction ¹³C' indicate a decrease in ¹³C content in agricultural sites. Errors are \pm 95% confidence intervals, n = 8. Overlap of the error bars with the dashed lines indicates no difference between native and agricultural soils. Two-way ANOVA summary comparing cropping systems is shown below the figure, with a complete ANOVA table is shown in Table S5.

3.4. Carbon storage capacity and the influence of texture

The total C concentration of the soils sampled here ranged from 1.5 g C kg⁻¹ in the native soil at 30–60 cm depth to 37 g C kg⁻¹ in the 0–15 cm depth interval of the cherry orchard soil. Over this C concentration range, MAOM C appeared to reach a maximum content of ~ 12 g C kg⁻¹ (Fig. 4a) and was more constrained than the POM C concentration, which increased linearly with increasing soil C concentration (Fig. 4b). In both fractions, the higher C concentrations were associated with the surface soil layers, where plant litter inputs and fine root turnover from shallow rooted grasses are greatest.

The amount of C in the MAOM was not strongly correlated with the amount of clay in the soils from either the native or the agricultural sites (Fig. 5). The range in clay content of the native site soils (2.5 to 20% clay) was less than that of the soils from the agricultural sites (2.5 to 69.5%), however, the majority of the agricultural sites (70%) had clay contents comparable to the native sites. In the native soils, increasing clay content appeared to have practically no influence on the MAOM C content, which ranged between 0.9 and 3.6 g C kg⁻¹. In the agricultural soils, MAOM C increased with clay content, but there was significant scatter in the data (Fig. 5). Fitted lines to both sets of data (soils from native sites and soils from agricultural sites) show low correlation coefficients; an analysis of covariance revealed that the intercepts (p < 0.003), but not the slopes, of the fitted lines differed between the two data sets.

3.5. Soil chemical and textural analysis

The textural classifications of these soils ranged from heavy clay to sand; of the 120 individual samples, however, most were classified as sandy loams, loams, silts loams and loamy sands (Fig. S1). The total organic and inorganic C content, total N, pH, texture and exchangeable cation concentrations of the soils sampled are shown in the Supplementary Data (Table S3).



Fig. 5. Relationship between clay content and MAOM C concentration in soils from native (filled circles) and agricultural (open circles) sites. Lines of best fit and correlation coefficients are shown for each site type.



Fig. 4. Relationship between concentration of soil organic carbon (SOC) and (a) MAOM and (b) POM carbon concentrations over three sampling depths for both native and agricultural sites, where filled circles are 0–15 cm soil depths, grey circles are 15–30 cm soil depths and open circles are 30–60 cm soil depths.

4. Discussion

4.1. Meta-analysis

The conversion of native lands for agricultural use typically results in a loss of soil organic C; a recent global *meta*-analysis estimated a median loss of 26% of the organic C from the top 30 cm of soil (Sanderman et al., 2017). Our *meta*-analysis of studies conducted in semi-arid regions with irrigation confirmed this general trend, and showed that C stocks have declined relative to adjacent native soils by 10.3% at the surface, and are largely unchanged below this depth. By contrast, we found that the soils beneath irrigated woody perennial crops in the semi-arid Okanagan Valley of British Columbia have gained C relative to native soils, with increases of 70% in C stock (25 Mg.ha⁻¹ to 30 cm depth), and of 100% in organic C concentrations in the 0–15 cm layer. Smaller increases in C stock of 10 Mg ha⁻¹ in the 0–30 cm depth have been previously reported for vineyards in this region (Holland et al., 2016).

4.2. Storage of soil C in POM and MAOM

By analyzing the C content of the POM and MAOM in these soils, our intention was to better understand the remarkable C gains that have apparently occurred by converting native sites to irrigated woody perennial fruit production in the Okanagan Valley. In most soils, POM typically only represents 10–25% of the total soil C content (Curtin et al., 2019). Gregorich et al. (2006) concluded from a review of over 100 publications and data from 67 agricultural sites, that POM contributes only \sim 21.6%, on average, of total soil C, although values vary widely (1.6 to 65.1%). Similarly, a recent, European Union-wide study of forests and grasslands, found that most C was stored in the form MAOM, particularly in soils with a low C content (Cotrufo et al., 2019). In marked contrast to these general trends, the majority of soil C in the Okanagan Valley soils is present as POM, not MAOM even though SOC is generally low. Fruit production has had a relatively small impact on the partitioning of C between POM and MAOM; in native soils at 0-15 cm depth, POM contributes 78% of the total C compared to 70% in agricultural soils. At 30-60 cm depth, this partitioning changes slightly but POM remains the dominant form of C, contributing 69% of the C in native soils and 62% in agricultural soils. Perennial fruit production increased the C concentration of both POM and MAOM fractions; at a depth of 0-15 cm, the MAOM fraction increased by 166% and the POM fraction by 80%. On average, the observed increase in soil C (10.01 g C. kg^{-1}) at 0–15 cm depth in agricultural sites relative to native sites was associated with a 63% increase in POM C and a 37% increase of MAOM C. At 30-60 cm depth, the soil C concentration increased by 2.5 g C. kg⁻¹; here, 49% of the C concentration increase was attributed to POM C, and 51% to MAOM C. These results partially confirmed our initial hypothesis, that POM is the dominant C fraction.

A number of factors may explain why most C is stored as POM and not MAOM in the soils across this region. One of the most important may be the mineralogical composition of the soils, specifically a lack of primary and secondary weathering products (Ross et al., 1985). Throughout the Okanagan Valley, glaciers retreating 10,000 years ago left a relative young, in geological terms, landscape (Fulton, 1969). Soils developed from glacial debris in this region support native plants with low natural productivity; this, together with the semi-arid climate means that only limited mineral weathering has occurred (Kelley and Spilsbury, 1949). Mineral weathering provides reactive surfaces capable of stabilizing organic C, thereby increasing soil C content and decreasing C turnover (Trumbore, 2009). At our native sites, relatively low amounts (<4 g C.kg⁻¹) of MAOM were measured regardless of clay contents, which varied considerably, and MAOM levels did not vary with depth in the soil profile. Irrigated agriculture significantly increased the overall MAOM C concentration, but the correlation with clay content was still poor, which was in contrast to our third hypothesis, and suggests that the availability of reactive mineral surfaces was not simply a function of clay content. This highlights the fact that not all clay-size particles are equivalent in their ability to stabilize SOC; instead this depends on a diverse range of mineral properties and their degree of weathering (Rasmussen et al., 2018). Increases in MAOM C concentrations in our agricultural soils were very likely the result of higher C inputs, as well as the impact of decades of irrigation, which has been shown to increase mineral weathering rates and clay illuviation (Presley et al., 2004). A similar effect has been observed in paddy field soils, where the cycle of flooding and drainage intensifies mineral weathering rates, and increases rates of organic C accumulation (Kögel-Knabner et al., 2010).

While on the one hand soil mineralogy constrained MAOM formation, confirming of our second hypothesis, the management strategies associated with perennial woody crops production promoted POM accumulation. In 2018, across the Okanagan region, cherry trees were 14 years old, on average (n = 16 sites), apple trees 22 years old (n = 29), and grapevines 17 years old (n = 21) (Midwood et al., 2020). Decades of minimal soil disturbance in these crops is conducive to POM accumulation. Particulate organic matter is known to be particularly sensitive to disturbance (Cotrufo et al., 2019), and the disruption of soil aggregates through tillage, for example, causes preferential loss of POM (Kögel-Knabner et al., 2008). Changes in management practices, such as the planting of a perennial bio-energy crop instead of annual crops, has been shown to increase POM-C by up to 71% over a 6-year period (Kantola et al., 2017). It is not only that the soil is left undisturbed for long periods in these cropping systems, but also that there is continuous plant C input into these soils from both long-lived crops and, in most cases, drive row vegetation, which may be planted or spontaneous natural vegetation. This continuous C input, may in fact be more important than the absence of tillage in the accumulation of soil C as POM (Mary et al., 2020).

4.3. Tracing C from litter to organic matter using stable isotopes

We used natural abundance stable isotope analysis to trace the flow of C from the plant litter into both POM and MAOM at our two site types. At both the native and agricultural sites, and across all depths, the POM was depleted in ¹³C content compared to the MAOM fraction. POM typically has an isotope value similar to the current standing vegetation (Del Galdo et al., 2003). The fruit trees, vines and most of the natural vegetation in the Okanagan Valley have a C₃ pathway of photosynthesis; tissue produced by C3 plants typically has a mean δ^{13} C value of -27‰, although this can range between -32 and -22‰ (Boutton et al., 1998). The relative enrichment of the MAOM δ^{13} C values compared to the POM, is due to isotope fractionation effects during microbial processing of the C entering this fraction (Menichetti et al., 2015). Much of the MAOM C is formed from microbial metabolites and necromass (Cotrufo et al., 2013); as the microbes metabolise components of the plant litter or turn over, ¹³C is retained, enriching the MAOM in ¹³C while the ¹²C is preferentially lost through soil respiration (Boström et al., 2007). The MAOM δ^{13} C values increased with depth across all sites as a result of repeated cycles of microbial processing and progressive adsorption and re-sorption of the organic matter on mineral surfaces deeper in the profile (Kaiser and Kalbitz, 2012; Mikutta et al., 2019). The POM δ^{13} C values also increased with depth but here, changes in the nature of the POM are likely to be the cause; with increased depth, the POM fraction becomes older, and is composed of smaller and smaller fragments of plant debris that are increasingly chemically resistant to mineralisation (Lavallee et al., 2020).

The δ^{13} C values of both the POM and MAOM fractions had been affected by irrigated agriculture; both were significantly less enriched in surface soils at the agricultural sites compared to the native sites. There are at least three possible explanations; firstly, and most obvious is difference in amount of new C entering the soil, which was significantly higher in the agricultural sites compared to the native sites. This recently assimilated C is isotopically depleted compared to the soil C (Ehleringer et al., 2000) and leads to an accumulation of C at all depths in the agricultural sites that is at an earlier stage of decomposition than the C at corresponding depths in the native sites. Secondly, at the native sites, there may have also been a minor C contribution from C₄ grasses, such as bunchgrasses, and/or succulents with CAM photosynthesis pathway, such as cacti; all of which occur naturally in the Okanagan Valley, but are not present at the agricultural sites. The mean δ^{13} C value of these plants is -13% and ranges between -17 and -9% (Boutton et al., 1998). Thirdly, it is likely the plants at the native sites are subject to more drought-stress especially over the summer months than those at the agricultural sites. Drought stress leads to an enrichment of 13 C within the plant tissues, as the leaf stomata close to preserve water, leading to a reduction in C isotope discrimination (Cernusak et al., 2013). Soil organic matter derived from these plants will be more enriched as a consequence.

At the agricultural sites, the difference in δ^{13} C values of the POM relative to the native sites followed a common pattern, declining to the 30 cm depth and then increasing again. Exactly why this occurred is unknown, but a notable difference between our agricultural and native sites was the fact that the woody perennial crops have roots that extend deeper into the soil profile than the natural vegetation at the native sites. It is possible that C inputs from the turnover of these deep roots are responsible for the δ^{13} C values from 30 to 60 cm at the agricultural sites. Similar results were observed in a ¹³C labeling study in a mixed conifer forest, where δ^{13} C value of SOC increased at depth; there, it was attributed to root inputs and reduced microbial populations available at depth to process these C inputs (Hicks Pries et al., 2018).

4.4. Implications for agriculture in this region

In general, at the agricultural sites few differences existed among the crop systems, contrary to our initial hypothesis; higher C input in the cherry soils led to significantly more POM C than in the soils beneath grape vines, but neither was different from the apple orchards. The MAOM δ^{13} C of the soil in cherry orchards was also more depleted, presumably due to higher C inputs, than in apple orchards irrigated by micro-spray, but neither were different for apple orchards irrigated by dripline or vineyards. Previous work in this region, using a larger dataset, found differences among these crops in the total amount of soil C storage; the highest soil C contents were found in cherry orchards (70 Mg C.ha⁻¹ to a depth of 30 cm) and the lowest soil C contents were found in vineyards (48 Mg C.ha⁻¹ to 30 cm) (Midwood et al., 2020). The results presented here suggest both POM and MAOM fractions respond to increasing C inputs in these woody perennial systems, but there may be a limit to C accrual as MAOM. A maximum concentration of around 12 g C kg^{-1} across the range of SOC concentrations measured, 1.5 to 37 g C kg⁻¹ was noted. A similar limit or inflection point for MAOM C concentrations was observed by Cotrufo et al. (2019) in soils from the European Union; in that case, the limit was higher, at around 50 g C kg⁻¹ soil, over a SOC concentration range of ~ 5 to 120 g C kg⁻¹, reflecting the presence of more varied and weathered soils. An effective saturation point for the MAOM C means that once this limit is reached, further C accrual can only occur through POM accumulation, which is essentially unrestricted (Cotrufo et al., 2019; Stewart et al., 2007). This has implications for the stability of the accrued C under irrigated agriculture in this region. For example, land use changes such as conversion to annual crop production would potentially reduce POM C concentrations, as a result of soil disturbance and tillage, and reduced C inputs during fallow periods in the growing cycle. Indeed, the establishment of vineyards on sites previously used for apple cultivation leads to a relatively rapid decline (over 9 years) in soil C stocks (Midwood et al., 2020); given the data collected in the present study, it seems likely the loss in soil C stocks associated with conversion from apple orchard to vineyard was driven by changes in POM. Returning agricultural land to a natural state would also lead to loss of POM C, since the C input rates from the native vegetation would be lower than under irrigated agriculture, negating any long-term storage of atmospheric CO₂ and climate change mitigation effects.

5. Conclusions

In contrast to most agricultural systems, including the soils of irrigated annual crops in other semi-arid regions, the soils beneath woody perennial crops in the Okanagan Valley of British Columbia accumulate significant amounts of C when compared to the adjacent native soils. By fractionating the soil into MAOM and POM, a more mechanistic understanding of these C gains was achieved. The C contents of both POM and MAOM fractions responded positively to cultivation, although the greatest percentage change was associated with the MAOM fraction. That being said, the MAOM fraction was not the dominant form of C in these soils and its formation is probably limited by the extent of weathering experienced by soil minerals across this region. Unlike many agricultural soils, most of the C in these soils was present as POM; this was also the case for the native soils sampled. Using natural abundance ¹³C analysis, differences in the formation of MAOM and POM, as well as the impact of higher C inputs due to cultivation were confirmed. The MAOM fraction was ¹³C-enriched relative to the POM as a result of microbial processing; whereas POM was more ¹³C depleted and presumably more similar to the C₃ standing vegetation. By establishing that POM is the dominant C fraction in these soils, a better understanding of observed rapid changes (over 9 years) in soil C content following the

conversion of apple orchards to vineyards across the Okanagan Valley was established. This observation also highlights the fact that although irrigated agriculture has led to the beneficial accumulation of soil C across this region, these C gains are vulnerable to changes in crop or management practices. To continue to benefit from higher soil C levels, improving agricultural productivity and helping to reduce atmospheric CO_2 levels, management practices which both promote and protect the POM in particular need to be adopted.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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