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Sources of soil carbon loss during soil density fractionation: Laboratory loss or seasonally variable soluble pools?

Ester Helbling, Derek Pierson, Kate Lajtha [*]

Dept. Crop and Soil Sciences, Oregon State University, Corvallis, OR 97330, USA

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ABSTRACT

To model global C budgets more accurately, we must better understand the dynamics and turnover of the many functional C pools that exist in soil. While soil density fractionation (SDF) is widely used to separate soil C pools based on the degree of stabilization by soil minerals, several studies have noted substantial losses of soil mass and soil C following SDF. As the source of these losses is unknown, they may lead to erroneous conclusions about SOM dynamics and inaccuracies in models of soil processes and global C cycling. For example, lab handling techniques such as air-drying soil could have an impact on soluble C losses. Alternatively, the observed C losses could represent pools of potentially soluble C that can vary by ecosystem and season, and thus might represent ephemeral, not well stabilized, and generally overlooked pools of soil C that are missing from SOM budgets and models. For example, in summer-dry climates such as the Pacific Northwest, soluble organic products of decomposition could accumulate over the summer when temperatures and microbial activities are high and precipitation is low, and subsequently leach during the fall and winter with precipitation. To address these divergent possibilities, soils were collected seasonally from a summer-dry forest in Oregon, and subsamples were subjected to 4 different laboratory handling procedures prior to fractionation: 1) air-drying and 2) oven-drying to simulate common laboratory drying techniques; 3) leaching to evaluate if potentially soluble C represented a pool that could be removed with simulated precipitation; and 4) immediate fractionation of fresh soil to determine if drying of soil caused artifacts in pools of potentially soluble soil C. Contrary to initial hypotheses, there were no seasonal trends to soluble DOC pools or total C loss during fractionation. Average mass loss during fractionation was 6% of initial dry weight and total C loss was 9% of total soil C. Soluble losses represented only 9% of total soil C loss. Particulate, or non-soluble mass loss was dominated by the high C:N free particulate organic matter, or LF. Thus, most C loss in this system was due to laboratory losses and that loss was biased towards an underestimation of LF matter in soil. DOC was a small enough component of loss that soil preparation prior to SDF was not significant. These results imply that in temperate soils, even in seasonally extreme eco-systems, relative proportions of mineral-associated organic matter and free particulate organic matter are seasonally robust, air drying of soil does not introduce error, and that immediate fractionation after field collection is not critical.

1. Introduction

Soil organic matter (SOM) is the largest store of terrestrial carbon (C), containing upwards of 1500 Pg of C globally in the top 1 m and 2,400 Pg C in the top 2 m (Scharlemann et al., 2014; Batjes, 1996). Given the large relative size of the soil C pool, small changes in either stabilization or destabilization of SOM can have profound effects on atmospheric C, global C cycles and global temperatures. Soil C flux to the atmosphere is an order of magnitude greater than fluxes from fossil fuels (Schimel et al., 2000), and thus an understanding of the susceptibility of different soil C components to destabilization is critical for models of the earth's C balance, as well as for management and use of terrestrial ecosystems. SOM is a complicated mixture of different functional pools of soil C that interact with microbes, temperature, moisture, and other biotic and abiotic factors to produce multiple pools with varying properties and turnover rates. To fully understand the underlying mechanisms involved in soil C storage and turnover, accurate methods are required to effectively separate SOM into specific pools based on similar

turnover rates and/or modes of stabilization.

Soil density fractionation (SDF) physically isolates soil particles based on the content ratio of OM to mineral matter in individual soil particles, taking advantage of the large disparity in density between OM and soil minerals. In wide practice, SDF is considered the best approach for separating free particulate, or light fraction (LF) materials from mineral-associated organic matter (MAOM), or heavy fraction (HF) matter (Sohi et al., 2001; Billings et al., 2020). LF matter, with a density lighter than 1.85 g cm^{-3}, is primarily unbound, partially decomposed plant residues. MAOM, or HF, with density greater than the LF, may be further separated to isolate unique portions of the HF material that may vary by mineralogy or degree of organic matter loading (Sollins et al., 2006). As intermediate decomposition products, LF has been found to be more labile than HF-SOM, with significantly lower turnover times, likely due to minimal physical protection mechanisms rather than inherent chemical recalcitrance (Baisden et al., 2002; Crow et al., 2007; Song et al., 2012). Exceptions to the more general pattern of lower turnover times in LF have been found when there is significant pyrolyzed C in the LF, which can alter radiocarbon dates but not mechanisms of C stabilization (Rasmussen et al., 2018; Baisden et al., 2002; Crow et al., 2007).

While SDF provides a widely suitable and robust method for separating soil C pools, a wide range of C losses resulting from SDF have been noted across various studies of soil C dynamics, with as much as 35% C loss shown in specific studies (Crow et al., 2007; Kramer et al., 2009; Throop et al., 2013). Such large and variable losses may greatly hinder the validity of further analyses and conclusions based on the SDF separated soil C pools. Losses of soil and C from SDF could be caused by many factors, including losses through handling, required laboratory procedures such as filtering, or simply the cumulative error inherent to a multi-step preparation and analysis procedure. The very large and consistent losses observed in several studies seem unlikely to be due to improper laboratory techniques, leaving open the possibility that much of the lost C is soluble and removed disproportionally during the SDF procedure, thus representing a real, and greatly under-represented soil C pool. Losses could also come from losses of colloidal matter or very fine clays, which would not be retained on filter paper or measured as DOC. However, if C losses from SDF are predominantly soluble, the amount of loss, and thus error in the analysis of soil C pools, may be further confounded by seasonal differences in soil C pools.

The idea that losses might represent a seasonally variable pool of soluble C is supported by a study of stream chemistry at the HJ Andrews Experimental Forest, which found that dissolved organic nitrogen concentrations in streams change throughout the year and peak just before maximum stream discharge in November-October (Vanderbilt et al., 2003). These authors hypothesized that this fall peak in dissolved organic matter was due to a fall rain flushing of decomposition products that accumulated over the summer months. Similar results were found in a study conducted on the Snake River in Colorado where dissolved organic C peaked before the peak in the hydrograph (Hornberger et al., 1994). Further, this same seasonal trend was seen in soil from a temperate grassland in California (Schaeffer et al., 2017), implying that there is wide potential for the existence of a mobile pool of C that is seasonally variable. Yet, the size and variability in this soluble soil C pool relative to total soil C has not been well quantified, and thus its relevance to SDF remains unknown.

The purpose of this study was to examine the losses of C during SDF and determine if the loss is (1) predominantly an artifact of lab handling and preparation processes, and if losses result from a specific C pool, thus limiting the usefulness of SDF as a mechanistic tool, or if losses (2) represent a real pool of potentially soluble and thus highly labile C which is seasonally and/or climate/ecosystem dependent and not well represented in current SOM modeling. We hypothesized that in seasonally dry environments, such as in Mediterranean summer-dry climates, potentially soluble C in the soil may be a significant fraction and source for C loss. In the Pacific Northwest, for example, summers are typically hot and dry: annual precipitation averages 250 cm with 80% of

that occurring between October – March (Vanderbilt et al., 2003). Soluble organic C is certainly created through the decomposition of OM via microbial and enzymatic activity throughout the year (Lajtha et al., 2005). However, we hypothesized that during the wet winter months soluble products are flushed through the soil profile, while during the summer months, when water flushing stops yet conditions remain favorable for microbial activity, soluble products accumulate in the soil. Thus, the pool of potentially soluble C should be greatest at the end of the dry summer. Similarly, pools of soluble C should be lowest in Pacific Northwest soils during the winter when the temperatures are low, limiting microbial activity, and heavy precipitation leaches any potentially mobile pool of C out of the topsoil.

To test our seasonal hypotheses, we collected soil from a summer-dry Pacific Northwest forest throughout the year to examine seasonal changes in soluble SOC. In addition, all seasonal soil samples were subjected to four laboratory treatments prior to fractionation to explore if lab handling techniques were responsible for any observed C loss. One subsample was immediately fractionated while field moist to avoid any artifacts from lab drying. Because it is a common practice to air-dry or oven-dry soil prior to soil analyses, subsamples of field-collected soil were subjected to two different drying treatments. One subsample was oven-dried for three days prior to fractionation. Another subsample was air-dried over three weeks, simulating slow drying that might occur at the end of spring and that we hypothesized might produce high levels of potentially soluble soil C. The final subsample was leached with a dilute solution of calcium chloride prior to fractionation to simulate high leaching rates during winter months, which we predicted would result in very low levels of potentially soluble C.

We predicted that the leached treatment would have low amounts of soluble C as would field-moist soil samples collected during winter months. In contrast, we predicted that the air-dried treatment would have the most soluble C due to microbial processes that would continue over three weeks allowing the soluble pool of C to build up prior to fractionation, similar to field samples collected at the end of the dry summer.

2. Methods

2.1. Site description

The H J Andrews Experimental Forest is located in the Cascade Mountain range, 50 miles east of Eugene, OR. The vegetation is dominated by mixed old growth Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), big leaf maple (*Acer macrophyllum* Pursh) and western redceder (*Thuja plicata* Donn ex D. Don). Vine maple (*Acer circinatum* Pursh), pacific yew (*Taxus brevifolia* Nutt.), pacific dogwood (*Cornus nuttallii* Audubon ex Torr. & A. Gray)*,* huckleberry (*Caccinium spp.*), and sword fern (*Polystichum munitum* (Kaulf.) are prominent in the understory (Sollins et al., 2009). The soil, an Andic Dystrudept (Sollins et al., 2006; Dixon, 2003) according to USDA soil taxonomy (Soil Survey Staff 2014), is developed in an alluvial/colluvial fan that now forms a gently sloping surface about 20 m above Lookout Creek. Rocks upslope (the presumed source of the deposit) are mainly ash-flow tuffs, both welded and non-welded, andesitic/basaltic in chemistry. The forest experiences an average annual temperature of 8.7 °C (Sulzman et al., 2005) and approximately 2.1 m of precipitation annually. The forest has a Mediterranean climate consisting of warm, dry summers and cold, wet winters. About 80% of the annual precipitation accumulates from September – March.

2.2. Sampling methods

A 10 m × 10 m area was selected for sample collection based on its proximity to the low-elevation Detrital Inputs and Removal Treatments (DIRT) research, where the soils have been well studied and characterized (Lajtha et al., 2005). To ensure that soils were comparable across

different seasonal sampling events, 12 0–10 cm cores of mineral soil were collected in random locations from within each quarter section of the plot area and composited, leading to 4 composited soil samples for each sampling event. Collection sites were flagged to ensure that they were not resampled. Each of the 4 composited samples was sieved to 2 mm and homogenized prior to subsample treatments.

Sample collection took place during 3 times of the year in order to capture distinct moisture and temperature regimes. The summer collection took place in August when the site had sustained months of warm and dry conditions. The winter collection took place from December – March when the site had sustained prolonged periods of cold temperatures and peak amounts of precipitation. The spring collection took place from May – June when the temperatures had started to rise and precipitation had started to decrease. Samples were collected in the different seasons over three separate years.

2.3. Soil treatments

For each homogenized soil composite, a subsample was air dried to determine total soil C prior to fractionation. The remaining homogenized material was split into 4 subsamples and subjected to different in-lab treatments to simulate different field conditions and handling techniques (Table 1). The first subsample was fractionated within a few days of sample collection, without drying to reflect the in-field conditions. The second subsample was air-dried at room temperature in the dark over three weeks prior to fractionation. The third subsample was oven-dried for three days at a temperature of 50 °C prior to fractionation. The fourth subsample was leached with a 0.02 M solution of $CaCl_2$ to leach out potentially soluble organic carbon prior to fractionation in order to simulate seasons with high precipitation and low microbial activity. For this treatment, 50 g of sample were weighed into PVC cores with a glass wool filter on the end and 500 mL of the $CaCl_2$ solution was added gradually. The samples were drained overnight prior to SDF the following day.

2.4. Soil density fractionation

Each sample was fractionated into a light fraction (LF) and a heavy fraction (HF) according to the procedure outlined by Sollins et al (2009). Only two fractions were separated to limit error resulting from an increased number of fraction pools. The density used to isolate the fractions was 1.85 g cm^{-3}, chosen based on a study conducted by Sollins et al., (2006) who found that this density successfully isolated large particulate organic material (defined as LF that was retained on a glass fiber filter) from the mineral-dominated soil in soils from the H.J. Andrews Experimental Forest.

Gravimetric moisture was measured on each sample after treatment to determine the starting density of the sodium polytungstate (SPT) solution to ensure the soil to solution ratio was consistent across treatments. Eighteen grams of soil on a dry soil basis was weighed into a 230 mL centrifuge tube and 60 mL of SPT was added. Samples were mixed on a shaker table for 2 h and centrifuged at 3000 rpms for 10 min. The density of the SPT solution was measured to confirm that the target density of 1.85 ± 0.02 g cm^{-3} was reached. If the density did not fall within the target range, the appropriate amount of water or SPT was

added accordingly and samples were shaken for 30 min and centrifuged in the same manner as before. After centrifugation, the LF and supernatant fluid was aspirated from the centrifuge tube and filtered through a Whatmann GF/C glass fiber filter paper. Samples were then rinsed with 1500 mL of Deionized (DI) water. The LF material was transferred with DI water to a drying dish and dried at 50 °C. The heavy fraction (HF) was rinsed in the centrifuge tube, shaken for 10 min, and centrifuged at 3000 rpms for 10 min. Rinsing was repeated until the supernatant fluid reached a density of 1.01 g cm^{-3} or less. The HF material was then transferred with DI water to a drying dish and dried at 50 °C.

A 10 mL aliquot of the soil SPT solution was collected right after the first spin in the centrifuge to capture the solubilized C released during the fractionation procedure. The samples were diluted 10 times using deionized water to reduce artifacts in the subsequent analyses from the SPT. The solution was then filtered using a Whatmann GF/C glass fiber filter paper and analyzed for TOC using a Shimadzu TOC-V analyzer.

The initial soil sample for total C analysis and the dried LF and HF post fractionation were ground to a fine powder using a mortar and pestle, dried in a desiccator, and analyzed for total C using a LECO analyzer.

2.5. Statistical analyses

Differences in means for LF, HF, and C component losses among treatments were tested with a linear mixed effects model. Sample preparation and season were initially used as fixed effects in the model, however season was not found to be significant and was subsequently removed as an effect for further study comparisons, which used a one-way analysis of variance (ANOVA). Assumptions of normality and constant variance of the residuals were checked graphically and appeared to be adequately met. Post-hoc Tukey honestly significant difference (HSD) tests were performed to find significant differences among pairwise combinations of treatments with a standard significance level of $p < 0.05$. All analyses were done with R version 3.5.2 (R Core Team, 2019).

3. Results

The mean dry mass of soil prior to fractionation was 18.2 ± 0.2 g and after fractionation the mean mass of HF and LF were 15.5 ± 0.2 g and 1.7 ± 0.1 g respectively (Table 2). The resulting average mass loss was 1.0 ± 0.1 g, or about 6% of starting dry mass. There were no seasonal trends for HF, LF, or total soil C recovery (or loss) either within or across treatments (Fig. 1). Over all seasons and all treatments, HF represented a mean of 36 ± 2% of total soil C, LF represented a mean of 55 ± 2% of total soil C, and losses represented a mean of 9 ± 2% of total soil C. There were no seasonal trends in the relative percentages of total C in any of these pools. Similarly, there were no trends in these pools by treatment.

The oven-dried treatment consistently produced more DOC across all seasons ($p < 0.0001$, Fig. 2). DOC made up an average of 9% of total carbon loss across all samples, and LF, or particulate C loss made up an average of 91% of total carbon loss.

4. Discussion

Amounts and relative proportions of recovered LF, HF, and total soil C did not vary seasonally. It was not surprising that fractions of C did not vary seasonally in this coniferous forest, as both above- and below-ground litterfall occurs year-round and the substantial litter accumulation at the HJ Andrews may be enough to replenish LF stocks throughout the year. Similarly, HF likely changes at a time scale significantly greater than a seasonal scale.

More surprising was the lack of seasonal variation in DOC loss from field collected soils. We initially hypothesized that as soils dry during the summer in a Pacific Northwest forest, microbial activity would be high and potentially soluble organic products of litter decomposition would accumulate in the soil without sufficient water movement for transport.

Table 1
Summary of laboratory subsample treatments.

Treatment	Description
Field Fresh	Fractionated immediately after sample collection
Air-dried	Dried at room temperature for 3 weeks prior to fractionation
Oven-dried	Dried immediately at 50 °C for 3 days prior to fractionation
Leached	50 g of soil was leached in a column with 500 mL of 0.02 M $CaCl_2$ prior to fractionation

Table 2

Total soil C, soil Light and Heavy fraction mass and C content, and total losses of mass and C during sequential density fractionation from soils collected from the HJ Andrews Forest during spring, summer, and winter.

| Season | Treatment | Total Soil Carbon | | Heavy Fraction | | | | Light Fraction | | | | Losses | | | |
| | | | | Dry Fraction Mass | | C in fraction | | Dry Fraction Mass | | C in fraction | | % Mass Loss | | % C Loss | |
		mg C/g soil	(SE)	mg/g soil	(SE)	mg C/g soil	(SE)	mg/g soil	(SE)	mg C/g soil	(SE)	%	(SE)	%	(SE)
Spring	Air-dried	43.83	(0.22)	865.30	(13.65)	20.03	(0.19)	74.83	(1.44)	21.59	(0.59)	6.01	(1.49)	5.08	(1.75)
	Field Conditions	47.00	(0.97)	848.27	(26.80)	18.63	(1.28)	79.56	(12.42)	23.20	(1.43)	6.20	(1.70)	8.92	(4.36)
	Leached	43.83	(0.98)	854.72	(17.25)	18.97	(0.55)	82.40	(4.26)	23.15	(0.88)	4.87	(1.42)	3.92	(1.84)
	Oven-dried	48.99	(0.96)	909.36	(2.92)	21.13	(0.02)	78.68	(10.95)	23.86	(3.63)	5.22	(1.13)	7.78	(1.51)
Summer	Air-dried	48.58	(0.06)	855.77	(15.44)	18.55	(0.43)	85.00	(8.45)	27.76	(1.65)	6.20	(1.06)	4.67	(3.51)
	Field Conditions	48.58	(0.06)	847.39	(11.17)	17.76	(0.83)	73.08	(6.25)	24.73	(1.44)	4.70	(1.14)	12.57	(2.10)
	Leached	54.30	(0.06)	790.34	(22.86)	13.86	(1.16)	161.47	(23.11)	39.55	(3.80)	4.27	(1.71)	1.61	(4.59)
	Oven-dried	54.32	(0.06)	855.14	(10.69)	17.54	(0.96)	103.92	(10.22)	33.56	(2.27)	3.93	(0.23)	5.26	(4.72)
Winter	Air-dried	43.44	(0.06)	880.11	(6.19)	15.11	(0.85)	81.10	(6.93)	24.70	(1.79)	3.02	(1.28)	8.84	(3.23)
	Field Conditions	44.07	(5.72)	850.62	(14.80)	13.14	(1.47)	94.29	(6.34)	26.45	(2.22)	7.11	(1.03)	12.67	(5.81)
	Leached	41.38	(6.28)	859.21	(15.08)	11.90	(0.33)	62.59	(5.66)	21.81	(1.46)	10.05	(1.08)	18.56	(3.14)
	Oven-dried	44.21	(5.42)	885.16	(7.64)	16.01	(0.68)	69.27	(7.51)	20.99	(3.24)	10.68	(0.12)	17.67	(6.66)
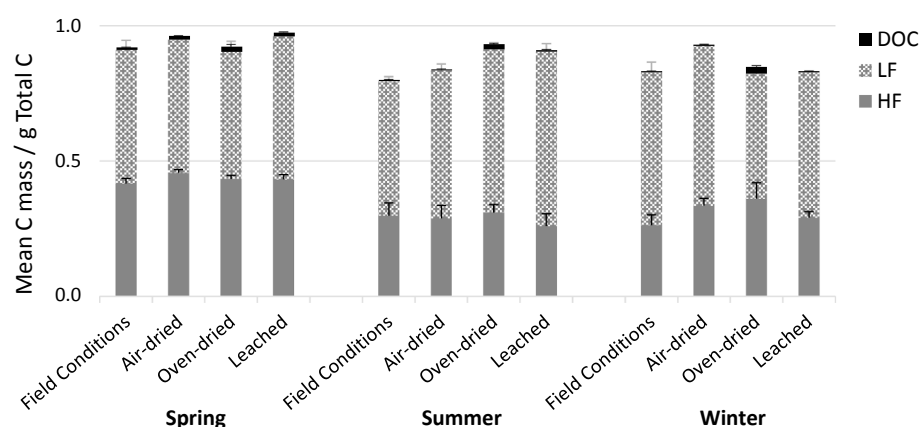
Fig. 1. Mean carbon mass of soil fractions (LF and HF) and DOC recovered from density fractionation as a proportion of the total soil carbon from treated subsamples collected from the HJ Andrews forest during spring, summer, and winter. Note that total soil C fraction mass is set at 1.0, and values of 1 – (LF + HF + DOC) represent unrecovered loss of C during fractionation. Values are means + SE.
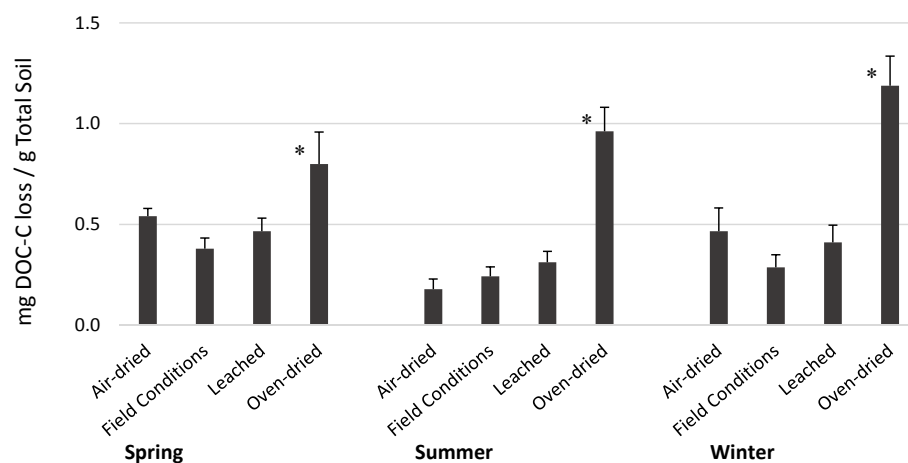

Fig. 2. DOC recovered from fractionating solution from the same soil collected at the HJ Andrews forest during spring, summer, and winter. Values are means ± SE. * represents significant difference of the oven-dried treatment across all seasons.

Thus, we expected that the DOC loss to solution would be greatest from soils collected at the end of summer. We also expected that the least amount of DOC loss would be seen from soil collected during the winter, due to lower microbial activity and persistent precipitation flushing soluble products further down the soil profile. The lack of seasonal variation in soluble DOC in this study is further surprising given that stream DOC data collected from the HJ Andrews and elsewhere lead to the hypothesis that soluble products do build up over the summer, or before snowmelt, and are flushed during the early fall with a peak in DOC before peak water flow (e.g., Hornberger et al., 1994; Vanderbilt

et al., 2003; Shao et al., 2015). One possible explanation for our results is that in summer, microbes not only produce potentially soluble products but also respire them, thus resulting in no net soluble C accumulation. However, if this production and consumption were evenly matched, local stream water DOC concentrations would be unlikely to display seasonal trends. The discrepancy between our results and the nearby stream water chemistry trends could perhaps be better explained by the fact that stream water reflects preferential flow in addition to bulk soil dynamics, and seasonal changes in stream chemistry can be linked to changes in the importance of preferential flow (Lajtha and Jones, 2018; Lee and Lajtha, 2016). Preferential flow paths have been shown to be enriched in microbial biomass and C and are "hot spots" for nutrient turnover and C mineralization (Bundt et al., 2001a, 2001b). Soil extracts are dominated by DOC released from shaken bulk soil, and it is possible that these bulk soil characteristics overwhelm subtle dynamics within preferential flow paths when soils are shaken and extracted.

The mean total mass loss after density fractionation was 6%. During fractionation, incomplete recovery of materials was unavoidable as there are losses with every transfer step, and the SDF process has many. For example, LF is rinsed on a glass fiber filter with 1500 mL of DI and then transferred to a glass drying dish using a squirt bottle of DI water, and brown coloring on the filter, no matter how much the filter is rinsed, indicates some LF is retained. Transfers of HF and LF during drying also involve loss.

There was a mean C loss of 9%, which is greater than the mean total mass loss, and soluble C losses were only about 9% of total C losses. Knowing the %C of both LF and HF of the total soil, and what had to be the %C of the lost material based on a mass balance, we calculated that the particulate material that was lost had a HF:LF ratio of 3.6, whereas in soil, the HF:LF mass ratio is closer to 9, implying a preferential loss of the high %C LF material. Alternatively, the preferential loss of high-C material might reflect colloidal loss, very small free particulate loss, or perhaps most significantly very fine class loss, none of which would not be collected on the filter paper or measured as DOC in the filtrate.

Studies in other ecosystems have reported greater losses with density fractionation, which could be due to different laboratory techniques, different vegetation characteristics, or different soil mineralogic properties. The soils at our site had andic properties and were derived from basalt weathering. Perakis and Hedin (2007) observed strong adsorption of low C:N hydrophobic materials by amorphous clays commonly associated with volcanic ash and basalt weathering, and Fujii et al. (2011) noted low DOC fluxes from Andisols compared to Inceptisols and Spodosols due to the high adsorption capacity of amorphous aluminum (Al) and iron (Fe) (hydr)oxides in the mineral horizons. Thus the absence of seasonality on DOC recovery from our soils could be due to laboratory shaking soils with highly adsorptive minerals; seasonality in stream water chemistry could be due to changes in preferential flow through andic soils that does not interact with soil minerals.

We initially expected lowest DOC losses from the leached treatments because we predicted that leaching would remove all labile DOC from the soils. We also hypothesized that air drying of soils would result in the formation of readily soluble C compounds due to prolonged microbial activity during the drying period. However, our data revealed the oven-dried soils produced the most DOC in the SPT fractionating solution, possibly due to heat-induced microbial lysis (Shehadul et al., 2017). While we did not heat samples higher than 50 °C, the 3 day oven drying procedure might have been long enough to denature the cell membranes and contribute additional soluble C. Throop et al. (2013) dried soil samples at 60 °C prior to SDF, which might be one contributing factor to the high losses seen in their study. If cell lysis is the mechanism behind the additional C measured in the oven-dried samples, then studies utilizing this method of pre-treating soils may be overestimating the soluble fraction of C in soil, although DOC loss was relatively small across all treatments for the soils used in this study. However, since oven drying soil has been shown to cause significant changes to estimates of extractable nutrients (Payne and Rechigl, 1989), oven drying of soil in

general should be avoided. In our soils, soluble DOC and estimates of LF and HF in the air-dried and field conditions subsamples did not vary from each other, demonstrating that soils can be air dried and stored prior to SDF.

Our results, taken together, imply that in temperate soils, even in seasonally extreme ecosystems, immediate fractionation after field collection is not critical (although oven drying should be avoided), and that relative proportions of mineral-associated organic matter and free particulate organic matter, or LF, are seasonally robust. Loss of DOC does not appear to be a significant pathway of C loss during SDF, but particulate C loss can be small but significant, especially from LF material that may be retained on filter paper, or from colloidal C that can pass through glass fiber filters.

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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