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## Electrostatic method to remove particulate organic matter from soil

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**TITLE:**

Electrostatic Method to Remove Particulate Organic Matter from Soil

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**KEYWORDS:**

soil, particulate organic matter, C:N ratio, soil analysis, light fraction, soil organic carbon

**SUMMARY:**

Removing recently deposited and incompletely decomposed plant material from soil samples reduces the influence of temporary seasonal inputs on soil organic carbon measurements. Attraction to an electrostatically charged surface can be used to quickly remove a substantial amount of particulate organic matter.

**ABSTRACT:**

Estimations of soil organic carbon are dependent on soil processing methods including removal of undecomposed plant material. Inadequate separation of roots and plant material from soil can result in highly variable carbon measurements. Methods to remove the plant material are often limited to the largest, most visible plant materials. In this manuscript we describe how electrostatic attraction can be used to remove plant material from a soil sample. An electrostatically charged surface passed close to dry soil naturally attracts both undecomposed and partially decomposed plant particles, along with a small quantity of mineral and aggregated soil. The soil sample is spread in a thin layer on a flat surface or a soil sieve. A plastic or glass Petri dish is electrostatically charged by rubbing with polystyrene foam or nylon or cotton cloth. The charged dish is passed repeatedly over the soil. The dish is then brushed clean and recharged. Re-spreading the soil and repeating the procedure eventually results in a diminishing yield of particulates. The process removes about 1 to 5% of the soil sample, and about 2 to 3 times that proportion in organic carbon. Like other particulate removal methods, the endpoint is arbitrary and not all free particulates are removed. The process takes approximately 5 min and does not require a chemical process as do density flotation methods. Electrostatic attraction consistently removes material with higher than average C concentration and C:N ratio, and much of the material can be visually identified as plant or faunal material under a microscope.

**INTRODUCTION:**

Accurate estimates of soil organic carbon (SOC) are important in evaluating changes resulting from agricultural management or the environment. Particulate organic matter (POM) has important functions in the ecology and physics of a soil but it is often short lived and varies based on several factors including season, moisture conditions, aeration, sample collection techniques, recent soil management, vegetation life cycle, and others<sup>1</sup>. These temporally unstable sources can confound estimates of long-term trends in stable and truly sequestered soil organic carbon<sup>2</sup>.

Despite being well-defined, common, and important, POM is not easily separated from soil nor is it easy to measure quantitatively. Particulate organic matter has been measured as that which floats in liquids (light fraction, typically 1.4-2.2 g cm<sup>-3</sup>), or as that which can be separated by size (e.g., > 53-250 µm or > 250 µm), or a combination of the two<sup>3-5</sup>. Both size-based and density-based techniques can influence the quantitative and chemical outcomes of POM measurement<sup>4</sup>. A careful visual inspection of soil that has been size-fractionated using routine methods often reveals long, narrow structures like roots and slivers of leaf or stem that have passed through the screen. Simply removing these structures by hand has been shown to substantially reduce measurements of total SOC<sup>2,6</sup> but the method is notably subject to the diligence and visual acuity of the operator. POM separation from a soil sample as the light fraction during flotation in a dense liquid<sup>7</sup> does not capture all POM, and excessive shaking during the flotation process can actually reduce the amount of light fraction recovered from a sample<sup>8</sup>. Flotation requires many steps and exposes the soil to chemical solutions which can change the chemical characteristics or dissolve and remove constituents that may be of interest<sup>4</sup>.

Alternative methods for removing POM have been used to avoid or augment the use of dense aqueous solutions. Kirkby, et al.<sup>6</sup> compared light fraction removal using two flotation procedures to a dry sieving/winnowing method<sup>9</sup>. Winnowing was performed by passing a light current of air across a thin layer of soil to gently lift away the light from the heavy fraction. The dry sieving/winnowing is performed similar to the two flotation methods with regard to C, N, P, and S content; however, the authors suggest that dry sieving/winnowing produced “slightly cleaner” soils<sup>6</sup>. POM has also been separated from soil using electrostatic attraction<sup>10,11</sup> in which organic particles are isolated by passing an electrostatically charged surface above the soil. The electrostatic attraction method successfully recovered POM, referred to as coarse organic particles, from dried, sieved (> 0.315 mm) soils with statistical repeatability comparable to other methods of size and density fractionation<sup>10</sup>.

Here we demonstrate how electrostatic attraction can be used to remove POM of sizes ranging from visible to microscopic. Unlike other reported methods, electrostatic attraction of fine soil also removes a small portion of mineral and aggregated soil which is visibly like the remaining soil. Given our results to date, it is reasonable to assume that the removal of a small portion of non-POM soil will have no substantial effect on the downstream analyses; however, this assumption should be verified for a specific soil if large proportions of the total soil sample are being removed electrostatically. The methods and examples provided here were performed on silt loam loess soils from a semi-arid environment.

This method may not be suitable for all soil types but has the advantages of being quick and efficient in removing particulate organic matter too small to remove manually or by an air current. Process speed is important in reducing fatigue, ensuring consistency, and encouraging greater replication for better accuracy of conclusions. Additionally, the ability to remove very small particulates is important in avoiding bias toward soils with larger rather than small particulate sizes.

## **PROTOCOL:**

### **1. Soil preparation**

1.1. Collect soil samples to the desired depth. Thoroughly dry the soil at 40 °C or following lab-specific standard protocols.

1.2. Sieve the soil through appropriate-sized soil sieves to obtain approximately 10-25 g of sieved soil. Many studies use a 1-2 mm sieve. The amount of soil is based on the mass required for the downstream analyses and will impact the number of times the electrostatic removal step will need to be repeated.

1.3. Place the soil in a clean, dry metal or glass flat-bottomed pan that is large enough for soil to be spread thin (at least 20 cm in diameter). Gently shake the pan horizontally to distribute the soil evenly in as thin a layer as possible.

### **2. Charge an electrostatic surface**

2.1. Hold a 100 mm diameter glass or polystyrene Petri dish top or bottom in one hand and vigorously rub the outer surface with a clean piece of nylon cloth, cotton cloth, or polystyrene foam several times. Perform the surface charging away from the sample to prevent fabric fragments from being introduced into the sample.

2.2. Inspect the surface of the Petri dish to make sure it is clean.

### **3. Remove particulate organic matter**

3.1. Lower the charged surface to within 0.5 cm to 2 cm above the soil and move it horizontally to pick up as much particulate material as possible. Attraction to the surface can be noted visually and audibly.

3.2. When the Petri dish no longer attracts additional particles, move the dish away from the sample.

### **4. Clean the electrostatic surface**

4.1 Hold the charged surface over a collection dish and use a fine brush to transfer the

electrostatically attracted material from the Petri dish surface into the collection dish. A camel hairbrush works well.

## **5. Repeat until the yield of particulates decreases**

5.1 Repeat steps 2 through 4 until the number of organic matter particles being picked up decreases. Redistribute the soil sample by horizontal shaking of the soil pan to expose new material at the surface and continue electrostatic collection.

NOTE: The endpoint is arbitrary and depends on the judgement of the researcher. Inspection of the charged surface after exposure to the soil gives a visual indication of whether a significant amount of organic particulates are still being removed from the soil. The final products are soil with reduced particulate content, and concentrated POM containing a small amount of electrostatically removed soil.

## **REPRESENTATIVE RESULTS:**

The results presented here are based on the analysis of silt loam soils from agricultural sites in the Pacific Northwest (**Table 1**). Soils were collected to depths of 0-20 cm or 0-30 cm, dried at 40 °C, passed through a 2 mm sieve, and treated using a polystyrene surface charged with a nylon cloth.

The amount of soil electrostatically removed from a sample varied. About 1% to 6% of the total soil mass was removed (**Table 2**). In all cases the proportion of total sample C removed was greater than the soil mass removed. Also, the C concentration and C:N ratio of the electrostatically removed soil fraction was always greater than the remaining soil. These factors indicate that the method reduced the amount of incompletely decomposed organic substances.

The ambient conditions and the combination of materials used to produce the charged surface affected the results (**Table 3**). The electrostatic removal method is expected to be less effective in a more humid lab environment due to lower surface charges. All materials should be as dry as possible for the electrostatic process. Nylon is a good material for electrostatic charging because it is lint-free and, when used with polystyrene Petri dishes, should produce one of the greatest electrostatic charges<sup>12</sup>. Alternatively, some types of polystyrene foam work well in combination with glass. The combination of a glass dish and polystyrene foam removed a greater amount of soil and C than either glass(dish)/cotton or polystyrene(dish)/nylon combinations.

Regardless of the materials used for surface charging, electrostatic treatment removed a greater proportion of C from the soil and produced a sample with lower C:N ratio as compared to the forceps/winnowing method although differences were only significant with glass/foam. Comparatively, flotation was more effective than electrostatic treatment in removing concentrated particulate C from the sample as noted by the lowest C:N ratio of the remaining sample and greatest C:N of the removed fraction.

The electrostatic treatment can be repeated numerous times albeit the treatments will begin to remove greater proportions of soil due to diminishing amounts of particulates attracted to the dish surface. The effects of treatment endpoints were examined by collecting a series of three electrostatic samples one after the other from the same soil sample (**Table 4**). The first treatment collected the greatest amount of C and although the following two treatments collected less, both were still highly enriched in C compared to the remaining soil. The C:N ratio decreased in the removed fraction indicating greater proportions of soil to POM were removed with each successive step.

When performing the ES procedure using a polystyrene Petri dish, scratches on the polystyrene dish surface were visible, suggesting the possibility that C from the plastic dish could contaminate the soil samples. When the ES treatment was performed on washed, C-free sand using a polystyrene dish, there was no detectable C in ES fractions even after four repeated treatments on the same ES fraction (data not shown).

Finally, the amount of particulate material that could be electrostatically removed from the fine silt-size fraction that passed through a 53  $\mu\text{m}$  screen was tested on five silt loam soils (**Table 5**). The electrostatically removed fractions demonstrated very little enrichment of particulate organic matter. Microscopic inspection reveals that POM does exist in the <53  $\mu\text{m}$  fraction of these soils (**Figure 1**), but in very small quantities. If the fine soil fraction (i.e., <53  $\mu\text{m}$ ) contains very little POM, that fraction can be removed prior to electrostatic treatment to reduce the amount of soil being treated. Sieve the soil over a very fine sieve, such as 53  $\mu\text{m}$ . Remove the soil from the top of the sieve and place in the tray for the electrostatic treatment, or simply use the sieve as the tray for spreading the sample. Return the fine fraction (soil passed through the sieve) to the electrostatically treated soil prior to chemical analysis.

#### FIGURE AND TABLE LEGENDS:

**Table 1: Soils tested.** List of samples used to compare the electrostatic process for particulate organic matter removal.

**Table 2: Representative removal rates.** The amount of soil in the electrostatically removed fraction (Removed) and the remaining soil fraction reduced in particulates (Remainder) as a proportion of the total sample mass and as the proportion of the total sample C. Also given are the concentrations of C, N and C:N. The estimated POM C:N gives the calculated C:N of the Removed fraction in excess of the concentrations in the Remainder, which is presumably the C:N of the POM removed. Numbers in parentheses are standard error of the mean. Analysis of variance indicated that Removed was greater than Remainder for both C and C:N ( $p > F$  of less than 0.0001). Replicates indicates the number of sample replicates per value. The electrostatic separation was performed with a polystyrene dish charged with nylon cloth after sieving out the fine fraction (<53  $\mu\text{m}$ ).

**Table 3: Technique comparison.** Removal of particulate organic matter from Thatuna soil using electrostatic attraction (ES), manual removal of visible particles with forceps and air

(Forceps/winnow), and flotation on sodium iodide solution at  $1.7 \text{ g cm}^{-3}$ . Electrostatic attraction was performed with a polystyrene dish charged with a nylon cloth, or a glass surface charged with a cotton cloth or polystyrene foam. Glass/foam was also tested under humidified conditions. Manual removal of particulates was performed by gently blowing air over the surface of a thinly spread soil to move it to the side and removing the visible residue with forceps. Data are the mean of six replicates. Means followed by a common letter are not significantly different according to the Tukey test at the 5% level of significance.

**Table 4: Investigation of endpoints.** Results of three successive electrostatic treatments to remove particulate organic matter. Average of three samples from the Thatuna soil and one each from the Ritzville-R, Ritzville-E, Walla Walla-M soils. The soil fraction passing through a  $53 \mu\text{m}$  sieve was removed before electrostatic treatment and analyzed separately. Data are the mean of the six analyses with the standard error in parentheses. Analysis of variance produced  $p = 0.06$  for both C and estimated POM C:N. Letters in the C:N column show significant differences between successive treatments at  $p < 0.05$ .

**Table 5: Particulate organic matter in the fine soil fraction.** Test of electrostatic particulate removal on the fine fraction ( $<53 \mu\text{m}$ ) of five soil samples from wheat cropping systems. An analysis of variance of Removed versus Remainder was not significant for C and C:N. The difference in C:N was not consistently greater in the removed fractions.

**Figure 1: Visual identification of particulate organic matter.** Microscopy images of the NT-AW soil as (A) whole soil, (B) removed fraction on the charged polystyrene surface, (C)  $<53 \mu\text{m}$  soil fraction, and (D) material that floated to the surface of a water slurry of the  $<53 \mu\text{m}$  fraction soil. Images were taken with 50x or 100x magnification. Images collected across several different focal points were combined in ImageJ software<sup>13</sup> using the Stack Focuser plugin (<https://imagej.nih.gov/ij/plugins/stack-focuser.html>).

## DISCUSSION:

The electrostatic attraction method was effective in removing POM from the silt loam soils. The method described here is slightly different from Kaiser, et al.<sup>10</sup> which used a combination of glass/cotton. We treated all but the finest soil fraction and used polystyrene rather than glass due to the triboelectric difference, which for polystyrene/nylon is  $100 \text{ nC/J}$  compared to glass/cotton at  $20 \text{ nC/J}$ <sup>12</sup>. Glass and polystyrene foam have proven effective and convenient in more recent experience. The relative humidity of the storage area and workspace could be an issue at some locations during certain seasons of the year. The methodology presented here was conducted in a workspace with consistently low (20% to 30%) relative humidity. Temperature would not be expected to change electrostatic attraction independent of humidity.

From our experience with the soils used for this research, the  $<53 \mu\text{m}$  soil can be sieved out of the sample before using the electrostatic process. Removal of the fine soil fraction prior to the electrostatic process seemed to improve attraction of particulates to the charged surface. Additionally, our soils did not appear to have significant quantities of particulates in the fine soil fraction, as indicated by its low C:N ratio. The electrostatic process was not effective at removing

the organic particulates which were present in this soil fraction (**Table 5**). This might not be true of other soils.

Researchers need to consider whether they are willing to remove a small amount of mineral soil along with particulate organic matter. Theoretically, the non-organic particulate matter (mineral) soil and aggregates removed with the electrostatic fraction might be chemically different or be coated with organic matter of a different nature than the remaining soil sample that will be used for chemical analysis. If substantial amounts of mineral soil are being removed, a chemical comparison might be warranted.

Adequate removal of POM is an important process for soil C estimates. The electrostatic method has some advantages over other methods including dry removal and flotation. These advantages include the ability to remove very small particulates, reduce process time, and retain the POM fraction for additional analyses. This method may not be suitable for all soil types or ambient conditions thus researchers are encouraged to validate the method for their specific samples and conditions.

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#### **DISCLOSURES:**

The authors have nothing to disclose.

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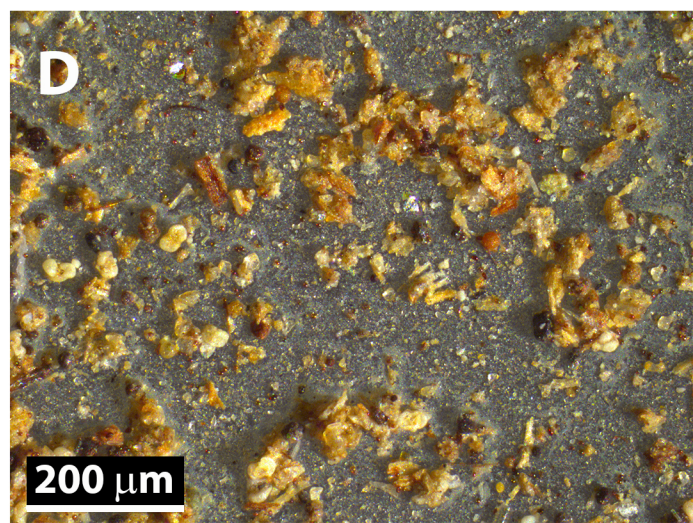
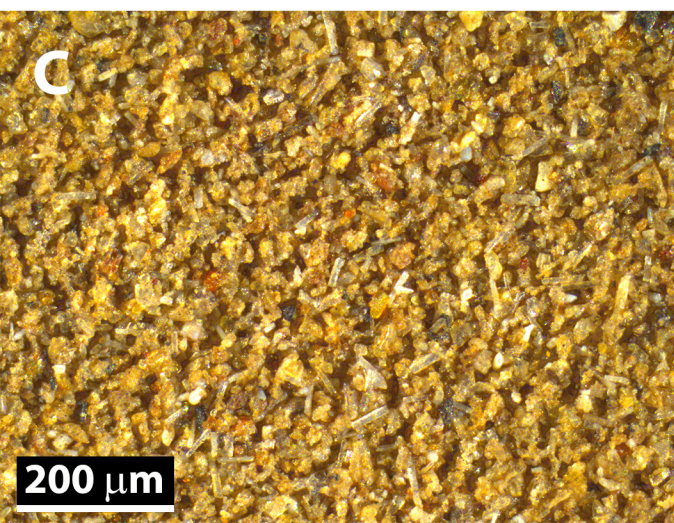
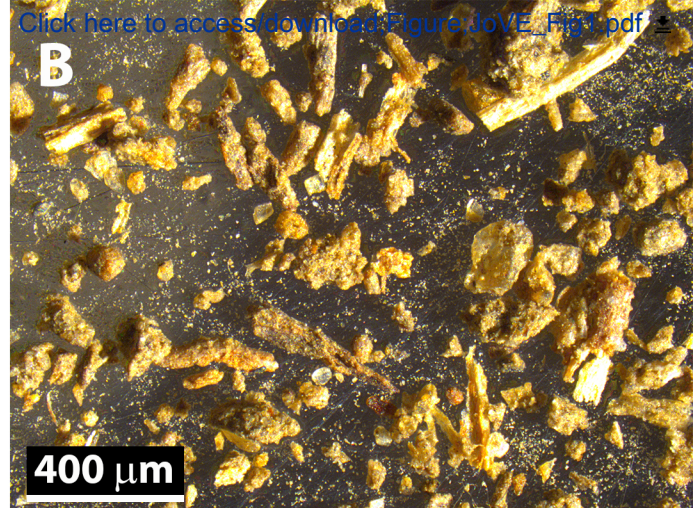
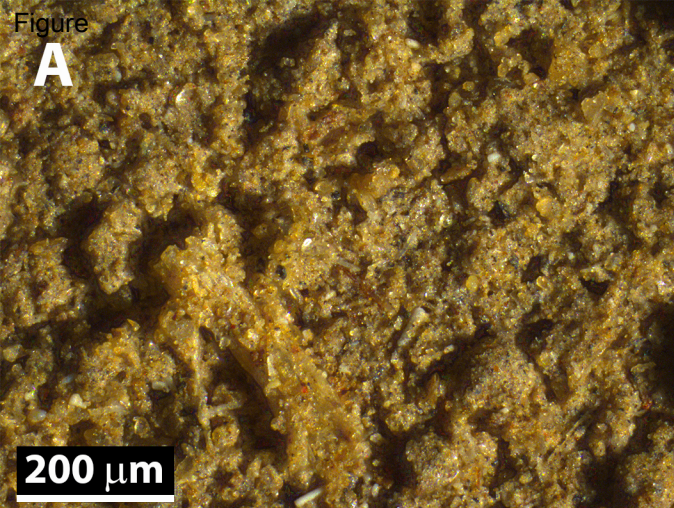


Table 1.  
Identifier  
Thatuna  
Ritzville-R  
Ritzville-E  
Walla Walla-M  
NT-AW

## Soil Type

Thatuna silt loam (fine-silty, mixed, mesic Xeric Argialboll)

Ritzville silt loam (coarse-silty, mixed, superactive, mesic Calcidic Haploxeroll)

Ritzville silt loam (coarse-silty, mixed, superactive, mesic Calcidic Haploxeroll)

Walla Walla silt loam (coarse-silty, mixed, superactive, mesic Typic Haploxeroll)

Walla Walla silt loam (coarse-silty, mixed, superactive, mesic Typic Haploxeroll)

Management	Collection depth	Mean Annual P Location
Wheat/fallow	0-30 cm	450 Pullman, WA
Wheat/fallow	0-30 cm	301 Ritzville, WA
Wheat/fallow	0-30 cm	290 Echo, OR
Wheat/fallow	0-30 cm	282 Moro, OR
No-tillage annual winter wheat	0-20 cm	420 Pendleton, OR

Table 2.

Soils/Treatments	Reps	Fraction	Proportion of total		C
			mass	C	
					g kg <sup>-1</sup>
Thatuna	10	Removed	0.01 (0.00)	0.05 (0.01)	54.02 (4.33)
		Remainder			14.52 (0.15)
Ritzville-R	5	Removed	0.02 (0.01)	0.08 (0.03)	36.24 (3.29)
		Remainder			9.61 (0.24)
Ritzville-E	8	Removed	0.02 (0.00)	0.07 (0.01)	36.73 (3.10)
		Remainder			7.31 (0.10)
Walla Walla-M	5	Removed	0.02 (0.00)	0.04 (0.00)	15.88 (0.55)
		Remainder			7.86 (0.05)
NT-AW	6	Removed	0.06 (0.01)	0.18 (0.02)	63.20 (9.25)
		Remainder			15.7 (0.31)

N	C:N	Estimated POM C:N
g-1		
2.85 (0.15)	18.68 (0.62)	24.39 (0.55)
1.25 (0.01)	11.58 (0.11)	
2.61 (0.21)	13.83 (0.16)	16.01 (0.15)
0.95 (0.01)	10.10 (0.18)	
2.65 (0.24)	13.89 (0.17)	15.94 (0.32)
0.78 (0.01)	9.40 (0.07)	
1.17 (0.04)	13.54 (0.21)	17.37 (0.91)
0.71 (0.01)	11.15 (0.20)	
3.81 (0.47)	16.32 (0.50)	19.75 (0.49)
1.40 (0.03)	11.21 (0.09)	

Table 3.		Proportion of total removed		
Method†		mass	C	
ES polystyrene/nylon	Removed	0.03 (0.01)	0.08 (0.01)	31.34
	Remainder			14.07
ES glass/cotton	Removed	0.04 (0.01)	0.10 (0.01)	28.20
	Remainder			14.12
ES glass/foam	Removed	0.08 (0.02)	0.13 (0.03)	24.59
	Remainder			13.95
ES glass/foam, humid	Removed	0.05 (0.01)	0.12 (0.02)	31.34
	Remainder			13.96
Forceps/Winnow	Removed	0.03 (0.01)	0.05 (0.01)	25.84
	Remainder			14.86
Flotation, 1.7 g cm3	Removed	0.01 (0.00)	0.10 (0.01)	141.28
	Remainder			13.19
Whole soil				14.50
† ES combinations are noted as the composition of the dish followed by the charging surface. Foam				

C		N		C:N
	g kg <sup>-1</sup>			
(4.21)		1.95 (0.15)		15.99 (1.07)
(0.35)	ab	1.23 (0.02)	ab	11.40 (0.18) ab
(2.32)		1.87 (0.13)		15.08 (0.49)
(0.32)	ab	1.23 (0.02)	ab	11.47 (0.12) ab
(2.85)		1.74 (0.11)		14.10 (1.11)
(0.20)	bc	1.20 (0.01)	bc	11.60 (0.15) ab
(4.58)		2.03 (0.20)		15.40 (0.75)
(0.36)	bc	1.23 (0.03)	ab	11.30 (0.13) b
(2.61)		1.61 (0.09)		16.10 (1.40)
(0.57)	a	1.25 (0.04)	a	11.90 (0.42) a
(15.63)		7.63 (0.62)		18.50 (0.58)
(0.58)	c	1.18 (0.02)	c	11.10 (0.50) b
(0.52)	ab	1.25 (0.02)	a	11.60 (0.44) ab

1 is polystyrene.

Table 4.

Fraction	Proportion of total		C	N g kg <sup>-1</sup>
	Mass	C		
1 <sup>st</sup> treatment	0.01 (0.00)	0.04 (0.01)	48.70 (6.67)	2.93 (0.41)
2 <sup>nd</sup> treatment	0.01 (0.00)	0.03 (0.01)	32.07 (3.56)	2.30 (0.28)
3 <sup>rd</sup> treatment	0.01 (0.00)	0.03 (0.01)	32.48 (4.68)	2.45 (0.40)
Remainder	0.60 (0.04)	0.60 (0.04)	12.02 (1.46)	1.11 (0.11)
< 53 µm fraction	0.37 (0.04)	0.03 (0.03)	9.51 (1.13)	0.96 (0.08)

S	C:N	Estimated POM C:N
0.27 (0.03)	16.6 (0.96) a	21.0 (1.88)
0.23 (0.03)	14.1 (0.63) ab	18.4 (1.89)
0.25 (0.04)	13.4 (0.46) bc	16.7 (1.29)
0.14 (0.02)	10.8 (0.29)	
0.11 (0.02)	9.7 (0.45)	

Table 5.

Soil/Treatment	Fraction	Proportion of mass	C	N	C:N
			g kg <sup>-1</sup>		
Ritzville-R	Removed	0.02	6.88	0.80	8.57
	Remainder		6.79	0.80	8.51
Ritzville-E	Removed	0.02	6.27	0.70	8.96
	Remainder		6.11	0.76	8.09
Thatuna	Removed	0.01	12.57	1.22	10.27
	Remainder		12.04	1.12	10.72
Walla Walla-M	Removed	0.02	7.33	0.75	9.75
	Remainder		8.01	0.84	9.56
NT-AW	Removed	0.01	13.13	1.25	10.47
	Remainder		13.77	1.30	10.58

Difference in C:N

0.06

0.87

-0.45

0.20

-0.12

Table of Materials

Materials	Company	Catalog
brush, camel hair	Any	
petri dish, glass or plastic	Any	
polystyrene foam, cotton or Any soil		
soil sieves	Any	

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#### [Editorial comments:](#)

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

*\* Done*

2. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step.

*\* The protocol was broken up into smaller steps where possible, and large paragraphs split or removed.*

3. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

*\* More detail was added, and the explanatory paragraphs moved to the discussion. I can't think of more detailed steps that would be logical to add.*

4. 1.1: Please add some details (as you have in the Representative Results and Figure legends) to have a specific detailed protocol that can be filmed. Information on the depth for soil collection can be moved to 1.1.

*\* How to collect the soil sample is not important to the method, ~~but we~~ made collecting and drying the soil sample the first step in 1.1, followed by sieving and suggested sieve sizes.*

5. Does temperature have any effects on the outcome of this separation technique?

*\* We did not test the effect of lab temperature, but I added that temperature effects would not be expected.*

6. Please add dimensions to the scale bars in the figures and also mention these in the legends.

\* **Kate?** *The scale bars have been standardized by length on all panels and the sizes are included in each panel. Since the dimensions now differ between panels and are noted on the images, we have omitted the size in the figure legend.*

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7. Please sort the Materials Table alphabetically by the name of the material.

\* Done.

#### Reviewers' comments:

##### **Reviewer #1:**

1) In the introduction section, although the authors point out that methods such as flotation have some obvious defects, the reason for choosing electrostatic attraction as a better way is inadequate.

*\*A paragraph was added to the end of the Introduction to outline the advantages, and another added to the conclusion.-*

2) Research method in the "Protocol" section should be described in more detailed, e.g. what was the size of the glass or polystyrene petri dish? Some basic physical and physical properties of the soil should be given. Ambient conditions and repeat times were considered and the results are presented in Table 3 and 4, while this is not mentioned in the "Protocol" section. In addition, descriptions of winnow/forceps and flotation methods and the analysis method for C, S and N, and should be introduced in this section.

*\*The ~~e-size of a typical the~~ petrie dish ~~size~~ was added to the protocol. The soil types used for examples are given in Table 1, and more details on minerology etc. are available on-line in soil surveys. The information in Table 3 is added to provide data to give an idea of typical results and how varying conditions and changing the protocol might affect results. ~~These comparative procedures are, but they are not part of the recommended standard procedures~~ recommended method of the study, therefore we feel they should not be included in the protocol section (but will upon editor request). ~~I don't believe the editor want comparison methods described in the protocol section.~~*

3) The data of the proportion of total mass and C seems not precise enough.

*\* These have a resolution of 1%, which is close to the average standard error of the mean. Fortunately, in this dataset they give adequate separation between the soils and treatments, and between mass and C.*

4) Why the C concentration of the removed soil in the flotation treatment was extremely high (141.28 g/kg)?

*\* Flotation is good at not including mineral particles, so it has a high C content. It does not necessarily remove all the POM, as shown in many studies. It did well in this case.*

5) From table 3, flotation was more effective than other treatment in removing POM, and the difference between the result of flotation and other method is relatively obvious. Discussion on which method is more reasonable should be given.

*\* I hope that the addition to the end of the introduction spelling out advantages of the electrostatic method will help address this. Some of the undesirable features of the flotation method are addressed in the second paragraph of the Introduction.*

**Reviewer #2:**

Minor Concerns:

The authors have presented a practical solution to a complex separations problem, i.e., removing particulate organic matter from soil using an electrostatic method. With minor corrections, this article is acceptable for publication in JoVE.

Line 166; line 170; Line 215; Table 3: Be specific if you are referring to "polystyrene" foam throughout the document.

*\* I have checked to make sure I used "polystyrene foam" everywhere it is used alone, but shortened to "glass/foam" in closely following references.*

The technique would be more robust if a wider variety of soils and a wider variety of humidities had been tested, but the authors have been forthcoming about these limitations.

Is the information regarding "Collection depth" (page 12 of the \*.pdf file) also a part of Table 1? Confusing. Oh, it must be part of the same table. The authors should have used a landscape orientation. Also, the data given for "Precip. (mm)" does not have a specified time period. Per hour? Per day? Per week? How does the precipitation relate to the time the sample was collected?

*\*Clarified to "mean annual precipitation"*

The authors should review and clarify their tables. For example, in Table 2, units are not given for mass. The "Proportion of total" heading spans two columns...but is not centered...is that meant to be? What do the numbers in parentheses represent...if that is supposed to be standard deviation, it should be defined at least in a footnote. Okay, I finally found the answer to this question in the Table legend. The "C" and "N" are reported in mass units...was "C:N" calculated as the ratio of molar units?

*\* C and N are in mass (g), and C:N is a mass ratio. This is typical for soil science.*

Lines 217-219: The explanation about the Tukey test is appropriate here, but it should also be added to Table 3 as a footnote. A table should be able to stand on its own.

*\* The editor can correct me, but I would assume that the table legends will be attached to the tables in the final printing.*

The style of the Tables will be up to the journal editor. However, I do prefer a (a) short title for each table, for example just the portion the authors denoted in bold print, (b) including the particulars as footnotes to the table, and (c) moving the discussion from the Table legends into the body of the text.

*\* I agree, but I was not sure what the journal would allow in the Excel version of the table.*

I thought it was strange to insert the "Figure and Table Legends" between "Representative Results" and "Discussion."

*\* ~~I thought it was what was called for in the instruction to authors~~ This is the JOVE template.*