**Response to Revisions**

**57464\_R1**

Improving Infrared Spectroscopy Characterization of Soil Organic Matter with Spectral Subtractions

Dear Editor,

Please find below detailing of revisions made in response to editorial comments. Please note that in additional to the changes noted below, as recommended by *JoVE* online instructions we have used this as an opportunity to correct the manuscript for additional clarity in grammar and spelling.

*1. The manuscript has been modified. Please read it carefully and revise if necessary. Enclosed please find the top copy. Please do not change the current format/font.*

The current format and font has not been changed. Revisions have been made as indicated below.

*2. Unfortunately, there are a few sections of the manuscript that show overlap with previously published work. Though there may be a limited number of ways to describe a technique, please use original language throughout the manuscript. Enclosed please find the iThenticate report. Please revise lines 280-282, 591-593, and the Figure Legend of Figure 1.*

These and other sections have been rewritten with original language.  *3. Please avoid sectioning the Introduction and Discussion sections.*

No sections were used. *4. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.  
  
5. 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.  
  
6. 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.  
  
7. For steps that involve software, please make sure to provide all the details such as “click this”, “select that”, “observe this”, etc. Please mention all the steps that are necessary to execute the action item. Please provide details so a reader may replicate your analysis including buttons clicked, inputs, screenshots, etc. This is the level of detail we’re looking for. Please keep in mind that software steps without a graphical user interface cannot be filmed.  
  
8. We cannot film the steps that are not fully described. Please do not highlight those steps which actions are only explained in the external references. Please keep those steps in the protocol but not highlighted. Please attention that your manuscript, after acceptance, will be the source for our script writers for the video production.  
  
9. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).*

All personal pronouns have now been removed.  *10. Protocol: 2.7: Oven-dry for how long?* This has been specified as follows:

“Oven-dry (60 °C maximum, 24-48 hours or as needed) to an air-dried state.”

*11. Protocol: 4.1.2: How to collect a background spectrum? Please clearly describe the actions.*

This protocol step (4.1.2) is the umbrella step for the ensuing specific steps on how to collect the background spectrum. Details on how to collect a background spectrum are provide in the 4.1.2.x steps. Please advise if this should be modified.

*12. Protocol:*[*4.1.2.1*](http://4.1.2.1/)*: How is that done?*

Since this is meant to a literal repetition of the loading technique described in detail for 4.1.1.3, this step is referenced as follows:

“4.1.2.1. Load a sample of KBr ground in the same manner as soil samples (see 4.1.1.3) to mimic soil matrix effects.”

*13. Protocol: 4.1.2.2 and*[*4.1.2.2*](http://4.1.2.2/)*: Please use the imperative tense for all the sentences.*

These have been rewritten in the imperative tense as follows:

“4.1.2.2. Collect background spectra and samples under the same ambient conditions. If

so, check changes in humidity and temperature mean that backgrounds and/or re-collect

the background spectrum.” *14. Protocol: 4.2.1: Please clearly describe all the actions in the imperative tense. Please avoid using any personal pronouns in the protocol steps. Please move the discussion to the Discussion.*

These have been rewritten in the imperative tense and personal pronouns have been removed:

“1.1.1. Subtraction factor: The subtraction factor (SF) weighs the degree to which absorbances in the mineral reference spectrum are subtracted from absorbances at the corresponding wavenumber in the original soil spectrum. For subtractions focused on improving resolution of organic absorbances to characterize SOM, it is recommended to utilize the entirety of the MIR collected by most spectrometers (*e.g.*, 4000 to 650 or 400 cm-1, depending on the detector).”

*15. Protocol: 4.2.2: Please clearly describe all the actions. How to use the software? Please include all the buttons clicked. Please move the discussion to the Discussion.*

We disagree with the recommendation to include details on the software used. This is because the procedure in question concerns spectral subtractions, which includes the pre-treatment for SOM removal and the adjustment of a subtraction factor. Whether this is used with a particular software program is arbitrary. Moreover, inclusion of software-specific steps could confuse readers who do not have this software, as FTIR softwares are highly manufacturer-dependent. We therefore believe the details specific to a software are best left out of the present method. To accommodate this, highlighting of this step has been removed so that the software-specific details of background spectra and sample spectra collection will not be in the video.

*16. Protocol: 4.2.2: Note: Please move this Note to the Discussion.*

This note has been removed since it is not relevant to the Discussion, but rather an observation that was originally thought to be of use to the reader by providing additional context.

*17. Protocol: 4.3: Please clearly describe all the actions in the imperative tense. Please move the discussion to the Discussion.  
18. Protocol: 4.3: Please clearly describe all the actions in the imperative tense. Please move the discussion to the Discussion.*

The actions have been rewritten in the imperative tense and the point intended to provide context has been moved to the Discussion, beginning at line 340:

“Since there is variability in OM and mineral types, as well as spectral artifacts from the method of packing, spectra of soils even with similar mineralogy and SOM content and removal may require slightly adjustment of the SF. If this is this case, Report the range of SF values used should be reported. If the same SF is applied across the spectral set, provide a justification. Such information is helpful to interpret results.”

*19. If possible, please shorten the length of the titles in the Figure Legends.*

This has been done for Table 1 and Figures 1 and 6.  *20. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file. Please list all the materials, equipment, instrument, and software used in your work.*

The table has been updated accordingly.