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ABSTRACT: Visible and near-infrared spectroscopy is a rapid, less expensive, and nondestructive alternative to conventional methods of soil analysis. This study aimed to investigate appropriate soil sample preparations and particle sizes for estimating soil organic carbon (SOC) through the use of laboratory spectroscopy. Rainfed paddy soils were sampled from 240 sampling sites to record their spectral reflectance and to measure their SOC contents in the laboratory. Partial least squares regression was applied to select the best model to estimate SOC using soil spectra. The results showed that the highest accuracy of SOC estimation was gained from soil samples prepared by 2 mm sieving. A short-wave infrared region was the most appropriate spectral wavelength for SOC estimation of rainfed paddy soil. Although the model showed potential in SOC prediction, the accuracy of partial least squares regression prediction in each spectral region varied between sampling times. Therefore, these models and methods should be further tested in soils sampled from different seasons and other regions to prove consistent validity. However, these results are useful for wavelength selection and soil sample preparation in future laboratory spectroscopy.

KEYWORDS: rainfed paddy, soil organic carbon, spectral reflectance, laboratory spectroscopy

Introduction

Soil organic carbon (SOC) plays important roles in soil qualities and carbon sequestration. Although the Walkley–Black method and the loss on ignition method have been well established as traditional methods for SOC analysis, these methods are time consuming, laborious, and expensive and result in soil sample destruction.[1,2] Therefore, methods for rapid, inexpensive, safe, and noninvasive SOC analysis, either in field or laboratory conditions, have been developed.[3] During the past 30 years, many researchers have proved that visible and near-infrared spectroscopy (VNIRS) can be used to estimate soil properties.[4,5] A current challenge for researches is to use spectroscopy for accurately predicting SOC without limitation of the amount of organic matter in soil,[6] in ways that are cheaper, faster, and more convenient than the conventional methods.[5]

VNIRS, usually in the range of 350–2500 nm, is widely used to measure an object's spectra for rapidly and accurately assessing soil attributes.[7] Depending on spatial scale and VNIRS measurement conditions, spectroscopy has been divided into three approaches: (1) laboratory spectroscopy, (2) portable field spectroscopy, and (3) remote spectroscopy.[8]

The laboratory domain has become well recognized before the field and remote domains.[9] Spectral signatures recorded by spectroscopy are defined by reflectance or absorption as a function of wavelength. However, the three approaches result in different spectra shapes, because of several factors such as atmospheric vapor, ambient light, parent material, soil composition, soil texture, soil moisture, soil temperature, soil surface roughness, and residue.[7,10]

Over the past 30 years, compared to spectral libraries of rocks and minerals, sharing of soil spectral libraries has been quite limited. There are no agreed standards or protocols for reliable reflectance measurements in the laboratory or the field.[11] The characteristic shape of soil spectra can be used to quantify soil attributes by matching with its soil properties measured by traditional methods to create an estimation model.[12] This method is efficient to predict SOC[13] and various other soil properties.[4] However, the relationship between SOC and reflectance is poorly correlated when soil samples are taken from large geographic areas with different parent materials or different landscapes.[14] Spectral signatures of paddy soil have not been widely shared because little research on spectroscopy

has been conducted in paddy fields. The paddy soil has a major role not only in economic crop cultivation in the Southeast Asia but also in environmental issues such as carbon sequestration.

Materials and Methods

Study area. The study site is located in rainfed paddy fields in Phitsanulok Province, in the lower northern part of Thailand (Fig. 1). Phitsanulok Province has a tropical savanna climate (Köppen climate classification, Aw) with an average annual temperature of 28.2°C and an average annual rainfall of 1,300 mm. Rainy season is from May to October.[15] Elevation ranges from 28 to 1,692 m, with lowlands in the west and higher elevations in the northeast. The deciduous and evergreen forests of the northeast cover 40% of the province's area. Total paddy areas of 2,805 km², of which rainfed paddy fields are approximately 1,200 km², cover 26% of the province and are mostly distributed in the central area.[16] In this province, there are five soil orders: ultisols, inceptisols, alfisols, entisols, and vertisols.[17]

Soil sampling. A total of 240 sampling sites with a plot size of 30 m² were randomly surveyed throughout rainfed paddy fields during harvested seasons, with the first field work (120 samples) in January 2013 and a second round of field work (120 samples) in December 2014. The soil taxonomy classification of all 240 samples, representing 40 soil series of Thailand, is shown in Table 1.[17] At each sampling site, a composite soil sample of top soil (0–15 cm) was taken with circular PVC plastic of 4-inch diameter at positions 1–9 using soil cores (Fig. 1).

Soil sample preparation. Soil samples initially collected in the field by using cores were air-dried in the laboratory at room temperature for one month before measurement of soil spectral reflectance on original dried-soil surfaces. After the first measurement, the surfaces of the samples were wiped off to remove crop residues before a second measurement of soil spectral reflectance. Finally, samples were grounded and sieved through three different sieving sizes (2.00, 0.50, and 0.07 mm) with spectral reflectance measured after each sieve.

Soil sample and statistical analyses. Only sieved soil was analyzed for SOC using the Walkley–Black method.[18] Analysis of variance was used to evaluate the difference of SOC contents between the first and second field works. At each wavelength of 1 nm interval in the range of 350–2500 nm, spectral reflectance measures from different soil sample preparations were analyzed using pair comparison. The correlation coefficient was analyzed to evaluate the relationship between SOC and spectral reflectance measured.
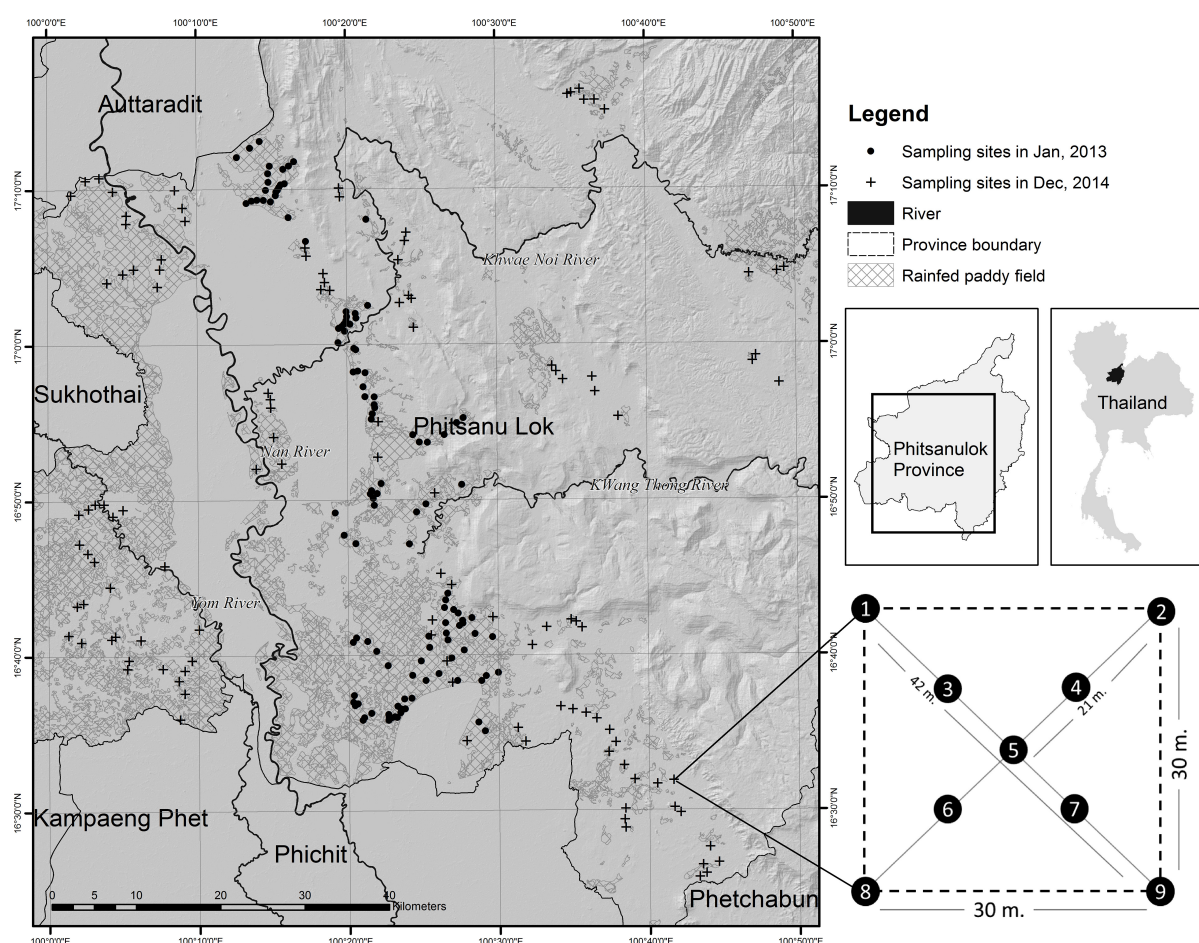

Figure 1. Study area and soil sampling.

Table 1. Soil taxonomy classification of all 240 samples.

SOIL FAMILY	SUBORDERS							
	AQUALFS	AQUEPTS	AQUERTS	AQUULTS	FLUVENTS	USTALFS	USTULTS	TOTAL
Clayey-skeletal, Kaolinitic, Isohyperthermic							2	2
Clayey-skeletal, Mixed, Isohyperthermic						1		1
Coarse-loamy, Mixed, Isohyperthermic					1			1
Coarse-loamy, Siliceous, Isohyperthermic	1					1	1	3
Fine, Kaolinitic, Isohyperthermic	1			1			2	4
Fine, Mixed, Isohyperthermic	3	1	1			2		7
Fine-loamy, Kaolinitic, Isohyperthermic							1	1
Fine-loamy, Mixed, Isohyperthermic	1			2	1		1	5
Fine-loamy, Siliceous, Isohyperthermic							4	4
Fine-silty, Mixed, Isohyperthermic	3					2		5
Loamy, Siliceous, Isohyperthermic						1		1
Loamy-skeletal over fragmental, Mixed, Isohyperthermic				1				1
Loamy-skeletal, Mixed, Isohyperthermic				1				1
Loamy-skeletal, Siliceous, Isohyperthermic							1	1
Very-fine, Mixed, Isohyperthermic			2					2
Very-fine, Smectitic, Isohyperthermic			1					1
Total	9	1	4	5	2	7	12	40

Spectral measurement. Soil spectral reflectance was measured in the laboratory dark room using a FieldSpec 3 ASD spectrometer as the setting instrument, as shown in Figure 2. Spectral reflectance measured at 1 nm intervals in the range of 350–2500 nm was recorded for 20 replicates per sample.

Spectral reflectance on the first field work samples was labeled as: (a) original soil surface, (b) wiped soil surface (a and b both remain contained in cores), and (c) 2.00 mm sieved soil (placed in Petri dish with 1 cm thickness; Fig. 3A–C). Spec-

tral reflectance of second field work samples was measured from three types of soil particle sizes placed in Petri dishes with 1 cm thickness, labeled as: (d) 2.00 mm sieved soil, (e) 0.50 mm sieved soil, and (f) 0.07 mm sieved soil (Fig. 3D–F). The original soil surface was influenced by residue cover, roughness, and soil aggregation. For wiped soil surface, the residues were removed. The effects of residues, surface roughness, aggregation, and particle size distribution were reduced for all the sieved soil samples.

SOC modeling. Partial least squares regression (PLSR), multiple linear regression for constructing predictive models in the presence of a large number of highly collinear predictors,[7] was used to generate the models implemented in the multivariate analysis software (The Unscrambler® X version 10.4 trial). By ascending sort of SOC contents, all data sets were separated for calibration (two-thirds of all data sets) and validation (one-third of all data sets). The 1 nm interval spectral reflectance was grouped into visible (VIS) at 400–700 nm, near infrared (NIR) at 701–1400 nm, short wave infrared (SWIR) at 1401–2500 nm, VIS-NIR at 400–1400 nm, NIR-SWIR at 701–2500 nm, and VIS-NIR-SWIR at 400–2500 nm. The best model was determined from low root mean squared error (RMSE) of validation, high coefficient of determination (R^2), and high ratio of performance to deviation (RPD).

Results and Discussion

Statistical analysis of soil samples. SOC measurements from second field work samples were significantly higher than those of first field work samples (Table 2). The amount of SOC
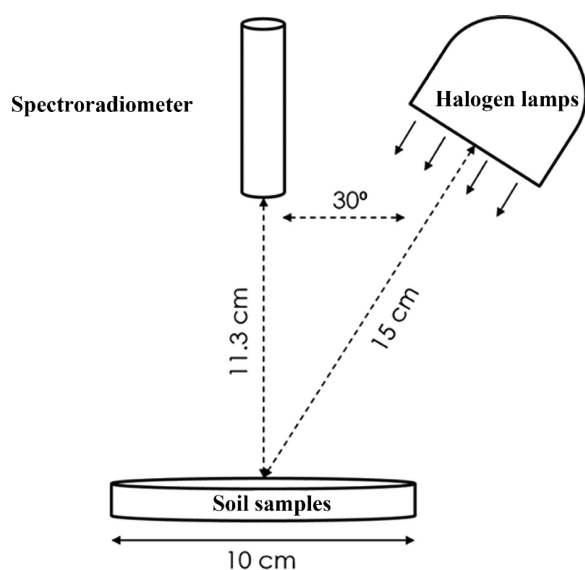
Figure 2. Soil reflectance measurement using spectroradiometer.

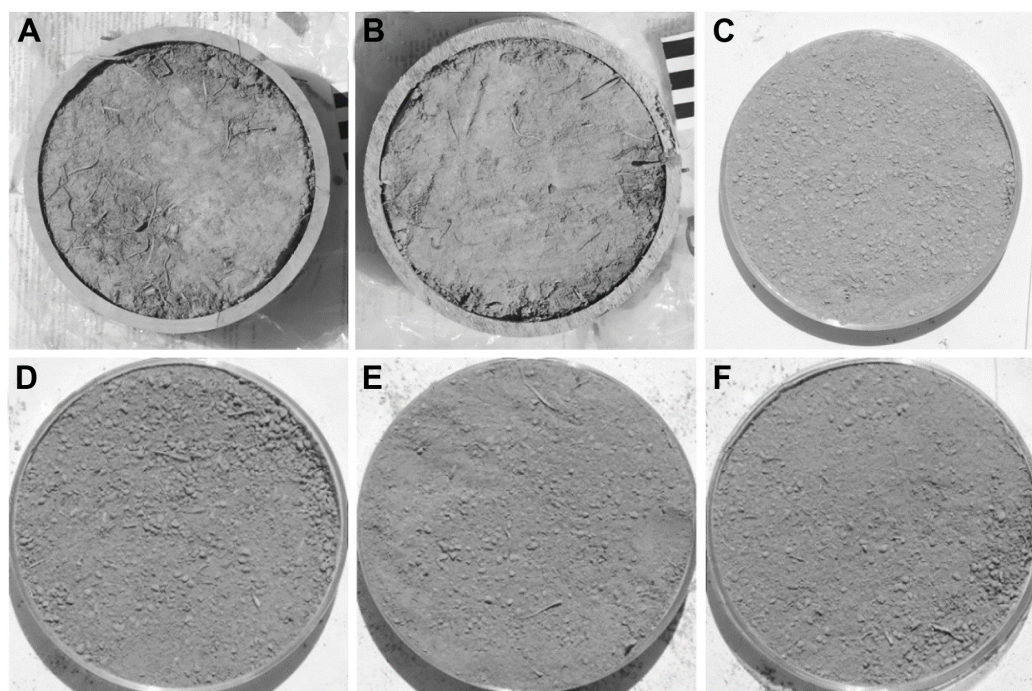**Figure 3.** Soil samples from first field survey (**A**) original soil surface, (**B**) wiped soil surface, (**C**) 2.00 mm sieved soil; and second field survey, (**D**) 2.00 mm sieved soil, (**E**) 0.50 mm sieved soil, and (**F**) 0.07 mm sieved soil.

in paddy soil in this study was similar to the findings of another study of paddy fields of Northern Thailand (0.43%–3.61%).[19]

Characteristics of soil spectral reflectance. Spectral signature showed an increasing pattern when wavelength increased, except three peaks at 1376–1424, 1918–1989, and 2141–2229 nm (Figs. 4 and 5). Spectral profiles increased sharply from 350 to 1385 nm and increased gradually in the range of 1385–2500 nm. These shapes of reflectance were similar to those of soil samples reported by Xiang et al.[20] Increased reflectance spectra were found in the 1100–2500 nm range, with three absorption peaks around 1400, 1900, and 2200 nm and a few small absorption peaks between 2200 and 2500 nm.[21] Absorption both at 1400 and 1900 nm was due to vibrations of water molecules absorbed in the minerals known as hygroscopic water.[11,22] Absorption bands were reported at 450–480 nm caused by hematite, limonite, and goethite.[23] The absorption at 1360–1490 nm, at 1810–1960 nm,[7] and at 1380, 1940, and 2250 nm was affected by hydroxide (OH) in free water and at 2200 nm by Al-OH lattice in clay minerals,[4,12,24]

2300 nm was assigned to carbonate,[11] and 400 and 2500 nm was absorbed by humic acid.[5]

Soil appears darker when SOC increases and this caused the lower reflectance.[5,25] Spectral reflectance of soil decreased when SOC increased (Figs. 4A–C and 5A–C). Sieved soil at 2 mm showed higher reflectance in both original and wiped soil (Fig. 4D), but showed lower reflectance for 0.50 and 0.07 mm sieved samples (Fig. 5D). Sieving and sieve size resulted in unsolid, homogeneous samples, size reduction of measured soil particles, and smoothness of soil sample surface, which can increase spectral reflectance at all wavelengths. Martinez et al[25] reported that decrease in reflectance with increasing surface roughness is possibly due to shadows cast on the surface and a greater proportion of diffuse scattering of light. Soil surface roughness can affect the relationship between spectral data and SOC. Spectral reflectance of fine-grained soil is greater than that of coarse soil.[26]

Effect of soil sample preparations on soil spectral reflectance. P-value indicated that reflectance of 2 mm sieved

Table 2. Statistical analysis of sieved soil samples.

SOIL PROPERTIES	PARTICLE SIZES	N	MEAN	STD. DEVIATION	MINIMUM	MAXIMUM
SOC (%)	0.07 mm (2nd)	120	2.57[b]	0.85	0.92	4.74
	0.50 mm (2nd)	120	2.39[b]	1.06	0.32	4.80
	2.00 mm (2nd)	120	2.38[b]	0.99	0.61	4.85
	2.00 mm (1st)	120	1.15[a]	0.73	0.43	3.61

Notes: [a]Different letters in the same column indicate significant difference ($P \leq 0.05$). [b]First and second were samples taken from first and second field works, respectively.
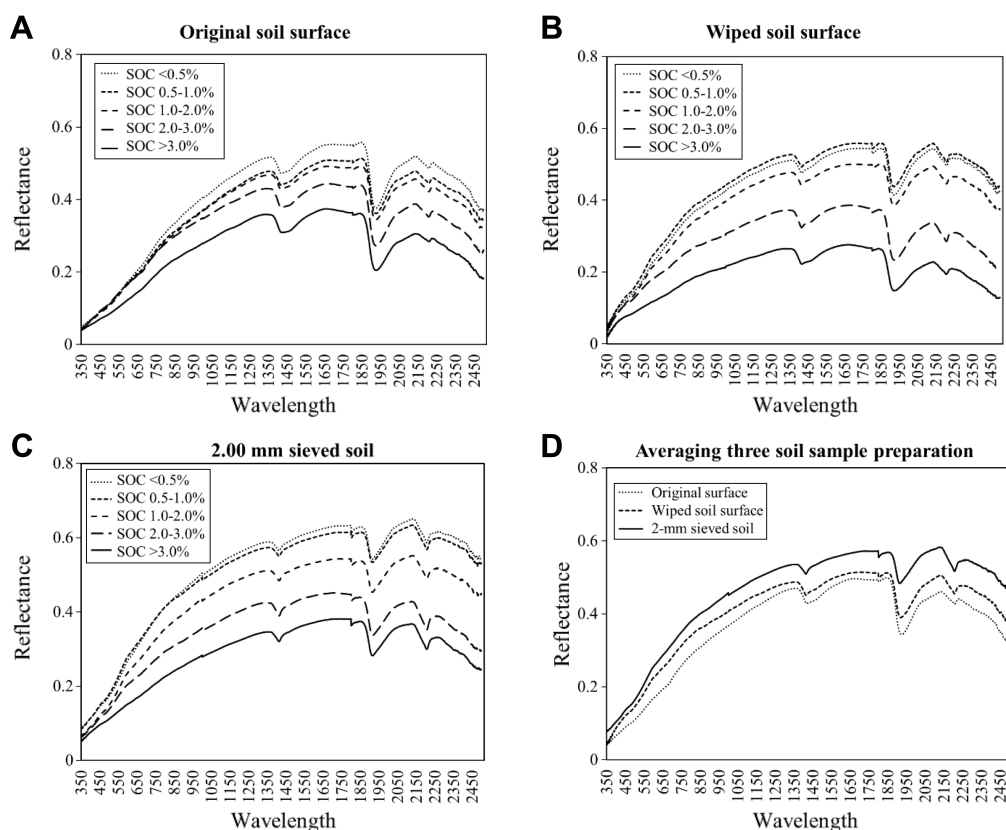
Figure 4. Spectral characteristics of samples taken from first field work: (**A**) original soil surface, (**B**) wiped soil surface, (**C**) 2.00 mm sieved soil, and (**D**) averaging three soil sample preparations.
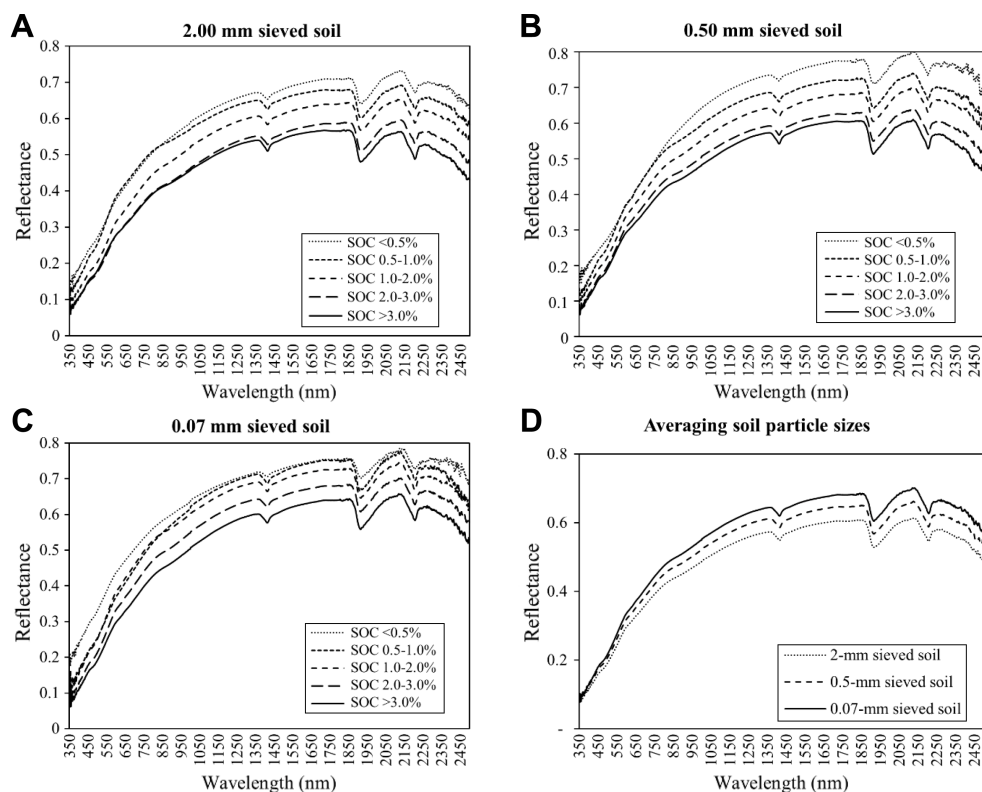
Figure 5. Spectral characteristics of samples taken from second field work: (**A**) 2.00 mm sieved soil, (**B**) 0.50 mm sieved soil, (**C**) 0.07 mm sieved soil, and (**D**) averaging three soil particle sizes.
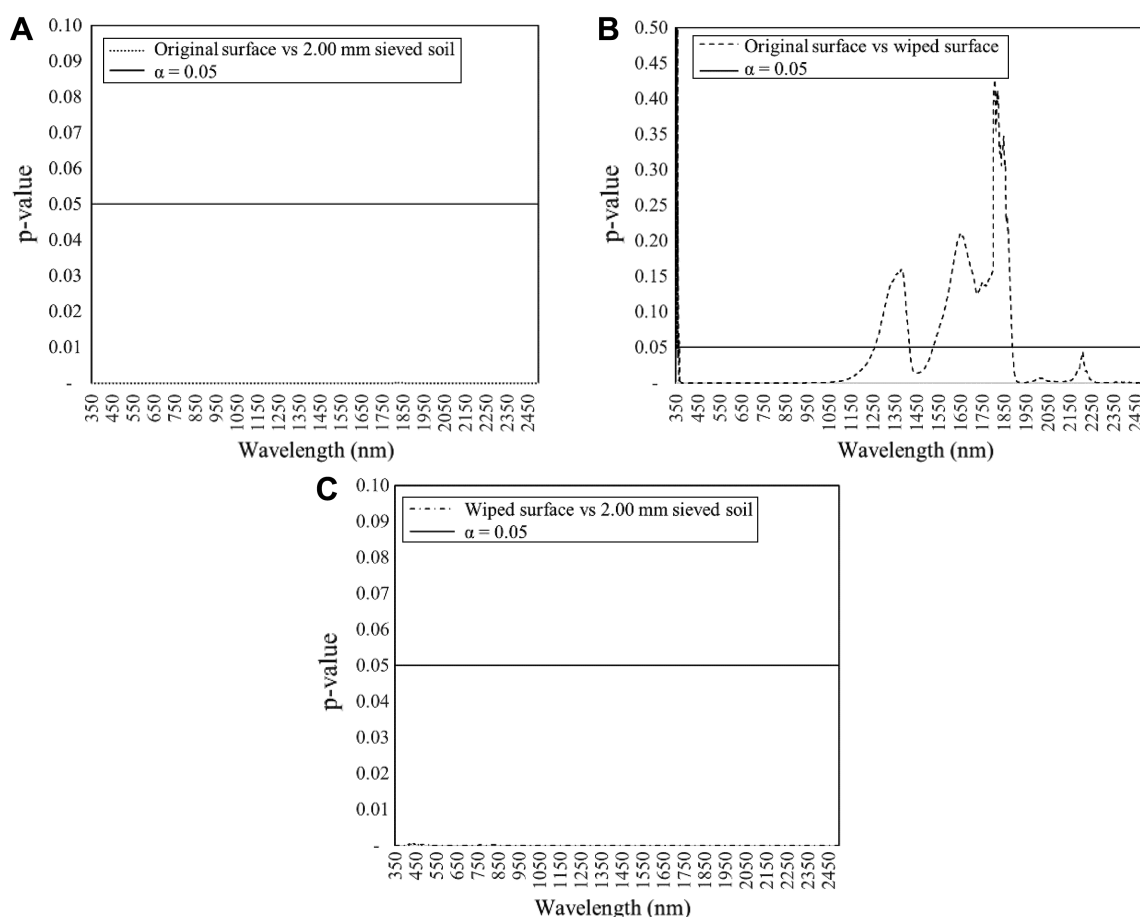
Figure 6. *P*-value of soil reflectance (**A**) between original surface and 2.00 mm sieved soil, (**B**) between original surface and wiped surface, and (**C**) between wiped surface and 2.00 mm sieved soil.

soil was significantly different from original soil and wiped soil for all wavelengths (Fig. 6A and C), while reflectance of original soil and wiped soil (Fig. 6B) were significantly different for some VIS regions (360–1115 nm) and SWIR regions (1930–2500 nm). The sieving process changes the physical properties of soil from solid soil to unsolid soil, resulting in a good penetration of energy source of reflectance. Therefore, sieved soil with finer particles showed higher spectral reflectance.

Sieved soil had stronger negative correlation compared to original surface and wiped surface samples (Fig. 7A). Finer particles also showed stronger negative correlation than coarser particles (Fig. 7B). SOC had a strong relationship with spectral reflectance in SWIR, NIR, and VIS. Nawar et al[27] found that reflectance in VIS (425–695 nm) had the highest correlation with organic C content among soils with the same parent material. In addition, some researchers found
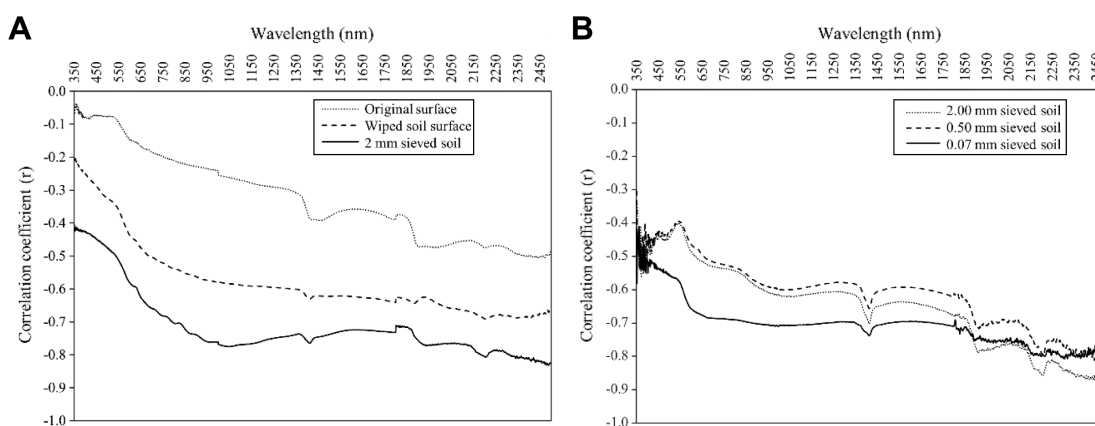
Figure 7. Correlations between SOC and soil spectral reflectance of soil samples considered on (**A**) three preparations and (**B**) three particle sizes.

that important VIS bands for SOC prediction were around 410, 570, 660 nm,[4] and 520, 540, 550 nm.[28,29] There are also strong correlations between OM and NIR ranges around 960, 1100 nm,[29] 1400 and 1900 nm,[30] 1720, 2180, 2309 nm[31] and 1744, 1870, 2052 nm.[32] Compared to NIR, MIR range provides better information about organic carbon in soil.[33] The most useful range to detect SOM was 2200–2500 nm, excluding

wavelengths between 2225–2255 and 2275 nm in order to minimize the effect of other soil properties. The 400–1100 nm range and some parts of the SWIR region (1100–2500 nm) might have potential to estimate levels of SOC sampled across large geographic areas on different parent materials.[14]

SOC modeling. As shown in Table 3, compared with SWIR and VIS, NIR had high R^2 in both the first and second

Table 3. SOC prediction using PLSR of first and second field surveys.

SAMPLE TYPES	SPECTRAL RANGES	CALIBRATION (N = 80)			VALIDATION (N = 40)		
		R^2	RMSE	FACTOR	R^2	RMSE	RPD
2 mm sieved soil*	VIS	0.72	0.31	6	0.69	0.33	1.83
	NIR	0.82	0.25	6	0.70	0.32	1.89
	SWIR	0.78	0.28	5	0.71	0.32	1.89
	VIS-NIR	0.79	0.27	6	0.72	0.32	1.89
	NIR-SWIR	0.82	0.25	10	0.75	0.29	2.09
	VIS-NIR-SWIR	0.81	0.26	4	0.74	0.30	2.02
	2161–2500 nm	0.81	0.26	9	0.73	0.30	2.02
Wiped soil surface*	VIS	0.59	0.47	8	0.66	0.42	1.74
	NIR	0.57	0.48	7	0.46	0.53	1.38
	SWIR	0.58	0.48	6	0.61	0.45	1.62
	VIS-NIR	0.59	0.47	5	0.47	0.53	1.38
	NIR-SWIR	0.62	0.45	9	0.70	0.39	1.87
	VIS-NIR-SWIR	0.60	0.47	6	0.52	0.50	1.46
Original soil surface*	VIS	0.29	0.61	9	0.36	0.58	1.26
	NIR	0.28	0.62	5	0.14	0.66	1.11
	SWIR	0.27	0.62	2	0.13	0.67	1.09
	VIS-NIR	0.41	0.56	6	0.14	0.67	1.09
	NIR-SWIR	0.30	0.62	2	0.16	0.66	1.11
	VIS-NIR-SWIR	0.38	0.58	6	0.16	0.66	1.11
2 mm sieved soil**	VIS	0.56	0.69	3	0.48	0.71	1.43
	NIR	0.63	0.64	5	0.74	0.51	1.99
	SWIR	0.72	0.53	2	0.81	0.44	2.30
	VIS-NIR	0.68	0.58	7	0.75	0.49	2.07
	NIR-SWIR	0.73	0.53	7	0.77	0.47	2.16
	VIS-NIR-SWIR	0.72	0.54	2	0.76	0.49	2.07
	2161–2500 nm	0.72	0.55	2	0.82	0.42	2.41
0.50 mm sieved soil**	VIS	0.49	0.73	5	0.51	0.71	1.42
	NIR	0.54	0.7	6	0.70	0.55	1.83
	SWIR	0.62	0.63	3	0.70	0.53	1.90
	VIS-NIR	0.58	0.65	7	0.58	0.65	1.55
	NIR-SWIR	0.59	0.66	2	0.68	0.57	1.77
	VIS-NIR-SWIR	0.60	0.63	3	0.70	0.50	2.02
	2161–2500 nm	0.62	0.62	3	0.74	0.51	1.98
0.07 mm sieved soil**	VIS	0.52	0.57	3	0.61	0.50	1.63
	NIR	0.61	0.5	5	0.61	0.51	1.60
	SWIR	0.66	0.48	2	0.59	0.51	1.60
	VIS-NIR	0.64	0.5	4	0.59	0.51	1.60
	NIR-SWIR	0.65	0.48	2	0.60	0.5	1.63
	VIS-NIR-SWIR	0.67	0.47	3	0.68	0.44	1.85
	2161–2500 nm	0.66	0.47	1	0.59	0.51	1.60

Notes: * and ** were samples of first and second field works, respectively.

field works. Also, the spectral combination of NIR-SWIR band resulted in higher R^2 than that of bands coupled with VIS. Based on a correlation coefficient >0.8, model accuracies of a selective band employed (2161–2500 nm) were improved in all evaluations. The R^2 values of SOC prediction models were 0.89 for MIR, 0.79 for NIR, and 0.74 for VIS.[5] Important wavelengths of VIS for SOC prediction were around 410, 570, 660 nm[4] and 520, 540, 550 nm.[29] Overall results, as shown in Table 3, indicated that sieved soil samples, particularly at 2.00 mm, showed a better performance for SOC predictions than those of wiped soil and original soil, respectively.

PLSR analysis (Table 4) was implemented only for the 2 mm sieved soil, by applying the highest accuracy model gained from the data set of the first field survey to the data set of the second field survey. The accuracies were obviously decreased because the range of validation data sets for the second field survey was greater than the calibration data set of the first field survey. In Table 5, when data sets of the first and second field surveys are combined to cover a wide range of SOC, the results show that a SWIR band was the most suitable range to estimate SOC, with accuracy at 74%. By removing absorption bands found in this study, model accuracies were not remarkably improved.

Conclusions

The spectral signature of rainfed paddy soil increased sharply from VIS to NIR and increased gradually from NIR to SWIR. Soil samples with low SOC content and fine particle size showed high spectral reflectance at all wavelengths. Sieving, particularly at 2.00 mm, showed high negative correlation coefficients with SWIR, NIR, and VIS. Predictive models calibrated by the PLSR method performed well, with coefficients of determination (R^2) > 0.60. The 2.00 mm sieved soil showed high performance for SOC estimation by using laboratory spectroscopy approach. An SWIR band is recommended for reliable estimation of SOC content, with validation R^2 > 0.70. Wavelength selection in narrow ranges or removal of absorption bands did not remarkably improve

model accuracies. Therefore, sieving at 2.00 mm was sufficient to prepare rainfed paddy soil samples for SOC estimation by using laboratory spectroscopy with SWIR as a principle wavelength region. Because accuracies of PLSR prediction varied between sampling time and among soil sample characteristics, further investigations are recommended in order to gain stable quality of VNIR applied for SOC estimation.

Author Contributions

Conceived and designed the experiments: SH, CN and WP. Analyzed the data: SH and CN. Wrote the first draft of the manuscript: SH. Contributed to the writing of the manuscript: SH, CN. Agree with manuscript results and conclusions: SH, WP, SL and CN. Jointly developed the structure and arguments for the paper: SH and CN. Made critical revisions and approved final version: SH and CN. All authors reviewed and approved of the final manuscript.

Table 5. SOC prediction of 2 mm sieved soil from combination of data set of first and second field works.

SPECTRAL RANGES	CALIBRATION (N = 160)			VALIDATION (N = 80)		
	R^2	RMSE	FACTOR	R^2	RMSE	RPD
VIS	0.73	0.53	8	0.73	0.56	1.86
NIR	0.68	0.6	10	0.60	0.66	1.58
SWIR	0.72	0.56	8	0.74	0.53	1.96
VIS-NIR	0.76	0.50	13	0.70	0.59	1.76
NIR-SWIR	0.73	0.55	8	0.72	0.55	1.89
VIS-NIR-SWIR	0.74	0.54	10	0.70	0.70	1.49
2161–2500 nm	0.63	0.66	3	0.68	0.58	1.79
NIR*	0.67	0.59	10	0.60	0.67	1.55
SWIR*	0.70	0.56	7	0.71	0.56	1.86
VIS-NIR*	0.75	0.51	12	0.70	0.57	1.82
NIR-SWIR*	0.70	0.56	8	0.69	0.57	1.82
VIS-NIR-SWIR*	0.74	0.52	10	0.69	0.58	1.79

Note: *Remove absorption bands at 1376–1424, 1918–1989, and 2141–2229 nm.

Table 4. SOC prediction of 2 mm sieved soil based on data set calibration of first field work.

SPECTRAL RANGES	CALIBRATION (N = 80)*			VALIDATION (N = 120)**		
	R^2	RMSE	FACTOR	R^2	RMSE	RPD
VIS	0.72	0.31	6	0.29	1.63	0.56
NIR	0.82	0.25	6	0.45	1.70	0.60
SWIR	0.78	0.28	5	0.61	1.67	0.63
VIS-NIR	0.79	0.27	6	0.45	1.63	0.62
NIR-SWIR	0.82	0.25	10	0.31	1.53	0.66
VIS-NIR-SWIR	0.81	0.26	4	0.51	1.54	0.66
2161–2500 nm	0.81	0.26	9	0.67	1.60	0.63

Notes: * and ** were samples of first and second field works, respectively.

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