

Exploring the feasibility of near-infrared spectroscopy in analyzing and predicting the chemical and biochemical changes of chitin-amended soils

Ken Abamba Omwange,¹ Ana Chen,^{2,3} Renfu Lu,¹ Carly Daiek,³ Julie Celini,³ Yan Liu³

¹USDA-ARS Midwest Area Sugarbeet and Bean Research Unit, East Lansing, MI, USA; ²School of Biological and Chemical Engineering, Anhui Polytechnic University, Anhui, China; ³Department of Biosystems & Agricultural Engineering, Michigan State University, East Lansing, MI, USA

Abstract

Chitin, a natural polymer, has been added to soil to enhance plant resistance to pathogens, yet its impact on soil's chemical and biochemical properties remains unclear. Standard methods to measure these properties are often slow and labor-intensive. This

study aimed to assess pH, total DNA, chitinase activity, and *trpB* gene DNA targeting for the identification of pathogen *Streptomyces scabies* in chitin-treated soil using both conventional wet chemical analysis methods and near-infrared (NIR) spectroscopy for rapid measurement. Five soil groups were treated with varying chitin levels (control, 0.2% and 2% fungus chitin, 0.2% and 2% Sigma chitin) and sampled every 9 days over 122 days. NIR reflectance spectra (900-1,685 nm) from the soil samples were acquired at each sampling time. Partial least squares regression models were developed using preprocessing methods like Kubelka-Munk, second derivative, and smoothed data. The second derivative model for 900-1,660 nm yielded high predictive accuracy with R^2 values of 0.915, 0.892, and 0.810 for pH, total DNA, and *trpB* gene DNA respectively. This study demonstrates the potential of NIR spectroscopy coupled with partial least squares algorithms as a rapid, cost-effective method for estimating soil biochemical properties.

Correspondence: Yan Liu, Department of Biosystems & Agricultural Engineering, Michigan State University, 1066 Bogue St #384, East Lansing, MI 48824, USA.
E-mail: liuyan6@msu.edu

Key words: soil property modeling; optical analysis; partial least squares regression.

Contributions: all the authors made a substantive intellectual contribution, read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Conflict of 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.

Availability of data and materials: data are available from the corresponding author upon request.

Disclaimer: mention of commercial products is solely for providing information for the reader and does not imply that USDA and MSU are endorsing them over those not mentioned.

Acknowledgments: the authors greatly appreciate the financial support from the United States Department of Agriculture, Agricultural Research Service (Award No: 58-5050-9-001). The authors sincerely appreciate the assistance of Dr. Yoshito Saito for his advice during data preparation.

Received: 17 July 2024.

Accepted: 4 December 2024.

©Copyright: the Author(s), 2025

Licensee PAGEPress, Italy

Journal of Agricultural Engineering 2025; LVI:1710

doi:10.4081/jae.2025.1710

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0).

Publisher's note: all claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article or claim that may be made by its manufacturer is not guaranteed or endorsed by the publisher.

Introduction

Soil provides nourishment to the plants, which is essential for the production of crops. However, soilborne pathogens and pests can cause great production losses, and they can persist in soil for many years. Chemical pesticides as one of the conventional pest control approaches are widely used in agricultural production systems. However, increasing concerns about the negative effects of pesticides and stringent regulations on their use call for new environmentally friendly pest control methods to replace these conventional chemical approaches. Biological methods such as biopesticides and biological control represent such an approach that will have minimal or no negative effects on humans and the environment. Different from conventional chemical pesticides, biological methods apply natural mechanisms against pathogens and pests. With increasing demands for clean (chemical-free) agricultural products, particularly organic products, it is expected that biological methods will be increasingly adopted for a wide range of cropping systems to protect crop production.

Chitin is a primary component of fungal cell walls and the exoskeletons of crustaceans and insects. Chitin can function as a plant disease vaccine to protect crops (Yin *et al.*, 2010). It has been reported that chitin and chitosan can induce resistance to plant pathogens (Reddy *et al.*, 1999; El Hadrami *et al.*, 2010), inhibit fungal and bacterial growth (Bautista-Baños *et al.*, 2006), improve seed quality (Zeng and Shi, 2009; Zeng *et al.*, 2010), and benefit wounded leaves recovery (Pearce and Ride, 1982). Besides, adding chitin made from shrimp and crab byproducts to soil can inhibit the growth of nematodes and fungal pathogens (Cardenas *et al.*, 1997; Kokalis-Burelle, 2001; Andreo-Jimenez *et al.*, 2021), and adding chitin in the soil can also promote chitin uti-

lization bacteria to generate chitinase for reduction of plant pathogens and insects (Chae *et al.*, 2006; Poulsen *et al.*, 2008). Thus, chitin could be used as a soil amendment material to protect crops. However, the effects of chitin on the chemical and biochemical properties of soil are not well understood and under-researched.

Conventionally, scientists rely on pH and deoxyribonucleic acid (DNA) measurements for the assessment of soil biochemical conditions. Soil pH gives the level of either alkalinity or acidity; for instance, slightly acidic soils provide optimum conditions for macro- and micro-nutrients availability (Brady and Weil, 2008; Ghehsareh and Namadi, 2012). Baig *et al.* (2020), reported that pH has proven to have a direct influence on soil nutrients, plant growth, and their development. Hence, soil pH has become an essential element or indicator for understanding the availability and weathering of soil nutrients and the relationship between soils and biota. With the use of polymerase chain reactions (PCR) for DNA tests, it can unravel the genetic information of organisms present in the soil by assessing their development and functioning (Nakatsu *et al.*, 2000). Therefore, pH and DNA can offer effective means for quantifying soil microbiological and chemical characteristics and provide independent analyses of changes in the soil's microbial communities' structures and functions (Kumar *et al.*, 2020, 2021). However, these two tests are costly (sampling methods and kits), require laboratory-based chemical analyses, and are time-consuming. Over the past two decades, near-infrared spectroscopy has been widely used as a quick and nondestructive analytical technique for soil composition and/or property analysis, because of its ability to relate the diffusely reflected radiation with various physical and chemical properties of soil (Chang *et al.*, 2001; Hummel *et al.*, 2001; Peng *et al.*, 2014; Xuemei and Jianshe, 2014). When soil samples are illuminated with visible and near-infrared light, certain bonds in the molecules vibrate with the changing electric field as the optical energy from vibration is absorbed thus reducing the amount of light reflected off the sample. While visible (Vis) spectra (400-700 nm) have been used to investigate the soil organic content with selected wavelengths, e.g. 564, 660, and 623 nm (Krishnan *et al.*, 1980), most soil-related diffuse reflectance studies have utilized the visible-to-near-infrared (Vis-NIR) regions between 400-2,500 nm (Blanco and Villarroya, 2002; Wang *et al.*, 2015), due to the existence of functional groups such as O-H, N-H, and C-H which are associated with the dominant absorptions peaks in this region (Blanco and Villarroya, 2002). Additionally, Vis-NIR reflectance spectroscopy has been used to evaluate the organic matter, moisture content, and cation exchange for surface and subsurface soils (Hummel *et al.*, 2001). However, to our knowledge, no studies have been reported on using Vis-NIR or NIR spectroscopy for measuring soil DNA and the microbial community of chitin-treated soil.

In this study, soil biochemical parameters namely pH, DNA, and pathogen DNA from five treatment groups with different levels of chitin were measured using the conventional laboratory-based chemical analysis methods. Subsequently, NIR reflectance spectra (900 nm-1,685 nm) were acquired from the same soil samples and pre-processed using the Kubelka-Munk (KM) and Savitzky Golay (smoothing and second derivative) methods. Partial least squares regression (PLSR) models were then developed using the pre-processed spectra data, to i) evaluate the potential of NIR diffuse reflectance in assessing the soil biochemical parameters; and ii) compare the performances of the PLSR models for the three pre-processing algorithms, namely KM, second order derivative and smoothing, in predicting the soil biochemical parameters.

Materials and Methods

Fungal chitin preparation

A filamentous fungus, *Rhizopus oryzae* (ATCC 9363), was cultured in the lab to generate biomass as the fungal chitin material following our previous work (Liu *et al.*, 2008). Modified Yeast-Peptone-Dextrose (YPD) was used as the seed medium, including 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, and 3 g/L CaCO₃. 1 mL spores suspension (10⁸/mL) was inoculated into 200 mL of the seed medium to produce seed broth at 27 °C, 180 rpm for 24 h. 10 mL seed broth was inoculated into 200 g of the fermentation medium to produce biomass at 27 °C, 180 rpm for 8 days. The fermentation medium contained: 200 g/L fresh potato, 5 g/L amylase, 1.5 g/L CaCO₃, 10 g/L peptone, and 5 g/L yeast extract. The mycelium was collected at the end of fermentation after autoclave and then soaked in 5% HCl for 15 min to remove the residue CaCO₃. The biomass was then washed with running water until pH neutral and lyophilized to generate dry biomass as the fungal chitin material. The glucosamine amount in the dried biomass was measured as the chitin content (Chysirichote *et al.*, 2014).

œ

The soil was collected from a potato farm located in Michigan (43.569220 N, 85.242350 W) and passed through a 10-mesh sieve to prepare the chitin-treated soil. Five groups of soil treatments were prepared, which included the lab-prepared fungal chitin (0.2% Fungus chitin/100 g soil, 2% Fungus chitin/100 g soil), commercial chitin (Sigma chitin Cat. No, 0.2% Sigma chitin/100 g soil, and 2% Sigma chitin/100 g soil and controls (the original soil). The moisture of the mixed soil was adjusted to 50% by adding autoclaved deionized water (DI) water.

Soil sample measurements

Laboratory chemical analyses for each of the five treatment groups in three replicates were done at 0, 9, 21, 30, 48, 60, 75, 91, and 122 days for the following measurements: i) soil pH: the pH was measured with a pH meter by saturating a 1 g sample with 2 mL ultra-pure water for 1 h. ii) Chitinase activities: 0.5 g soil was added into 1mL Phosphate-buffered saline (PBS) solution vortex mixing for 30 s before centrifugation at 10,000 rpm for 1 min; the chitinase activity of the supernatant was detected and calculated according to the recommended protocol in the Chitinase Assay Kit (Catalog #CS0980-1KT, Sigma-Aldrich, St. Louis, MA, USA). iii) Soil total DNA extraction: DNeasy Powersoil Pro kits (Catalog #47014, QIAGEN, Germantown, MD, USA) were used to extract DNA from the soil samples. The extracted DNA was stored at -80°C for future uses. The concentration of DNA was quantified using a fluorescent dye (Picogreen) method with a FLUOstar OPTIMA microplate reader (DR-200B, Diatek Instruments Co., Ltd, Wuxi, China). DNA standards with concentrations of 0, 1.25, 2.5, 5, 10 ng/μL DNA was used to generate the standard curve for sample DNA calculations. iv) Quantitative polymerase chain reaction (qPCR) for pathogen analysis: *trpB* gene, targeting for specific identification of potato pathogen *Streptomyces scabies* (Xu *et al.*, 2016), was monitored and quantified by a CFX Connect™ Real-Time PCR Detection System with SsoAdvanced Universal SYBR Supermix (Catalog# 1725272, Bio-Rad, Hercules, CA, USA). The sequences of the forward primer trpB280F, and the reverse primer trpB405R are shown in

Table 1. The primer concentration was optimized, and the best final concentration is 500 mmol/L. The total extracted DNA samples were normalized to 1 ng/uL as the templates before qPCR quantification. The optimized annealing temperature was 65°C. The other reaction conditions were performed according to the recommended protocol in the Bio-Rad SsoAdvanced Universal Kit. The *trpB* gene was used as a standard to construct a standard curve. The efficiency of the qPCR assay was 90-105% under the optimized conditions.

Near-infrared reflectance spectra measurement

Following the laboratory chemical analysis, the soil samples were scanned to acquire NIR reflectance spectra over 900-1,685 nm using an NIR spectrometer (NIR-512L-1.7T1:-10-USB/1.5/25um, Control Development, Columbus, OH, USA). For the five treatment groups, each soil sample was placed in a cylindrical PVC sample holder of 50 mm inner diameter and a height of 65 mm. The NIR light source was placed 60 mm above and perpendicular to the sample surface whereas the spectrometer's detecting probe was arranged 30° from the light source and 64 mm above the sample (Figure 1). Each sample was scanned three times, and a total of 135 spectral acquisitions (i.e., 5 groups × 3 replicates × 9 days) were measured in the whole experiment for analysis. For every sampling date, the blank/dark reflectance was acquired by turning off the light when scanning. A 40% certified reflectance standard disk (Labsphere, Inc., North Sutton, NH, USA) was used as a reference reflectance, which allowed the use of the same instrument measurement settings, without causing signal saturation to the NIR sensor.

Reflectance data pre-processing

After sampling, the dark measurement was subtracted from the sample and reference measurements to normalize the values to zero. Thereafter, relative reflectance spectra were calculated by dividing the dark-corrected sample reflectance by the dark correct-

ed reference reflectance multiplying by a constant value of 2.5. Multiplying the dark corrected reference reflectance by a constant value of 2.5 would be equivalent to generating a new reflectance that would have been obtained with a 100% standard reference. The relative reflectance data was then subjected to the KM pre-processing treatment to eliminate the backscattering effect (Alcaraz de la Osa *et al.*, 2020) from the soil samples, which mainly come from an infinite number of layers that cause absorption fraction and remission fraction. Furthermore, the Savitzky Golay smoothing and second derivative techniques were also applied to the relative reflectance data, respectively, to remove the scattering effect resulting from soil particles. During the analysis, two wavelength ranges were evaluated for regression analysis: whole wavelength (900-1,685 nm) and 900-1,660 nm (excluding reflectance >1,660 nm because of lower signal-to-noise ratio).

Data processing and modeling of the biochemical properties of chitin-amended soil samples

Partial least squares regression (PLSR) was conducted to assess the feasibility of using NIR spectra to determine the biochemical parameters (pH, total DNA, and *trpB* DNA) of the soil samples with different chitin treatments. MATLAB software (MATLAB 9.5 R2023b; The MathWorks, Inc., Natick, MA, USA) was used during the data processing. To establish and validate the calibration model, the data were divided into two sets: the calibration set (70%) and the prediction set (30%). A PLSR prediction model was established using the reflectance data pre-processed with each of the three techniques described above to predict the

Table 1. The primers of the *trpB* gene used for qPCR.

Primer	Nucleotide sequence (5'-3')
trpB280F	TGGGTCGCCAACGTGACCG
trpB405R	GCGGCGCCAGATCCTCGAACG

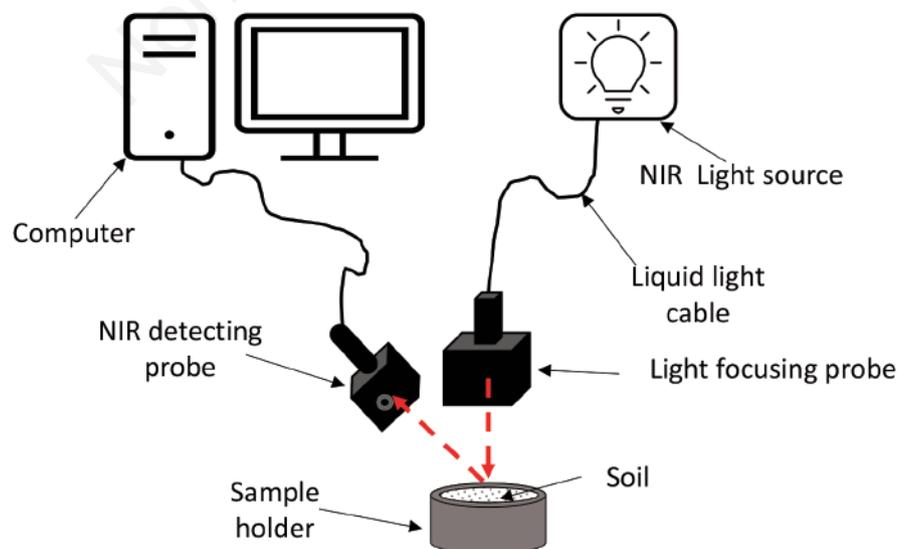


Figure 1. Near-infrared (NIR) instrumentation setup for soil reflectance measurement.

pH, total DNA, and *trpB* DNA of the soil. The model performance was evaluated using the coefficient of determination for validation or prediction, R^2_{pred} (Eq. 1), the root mean squares error of prediction $RMSE_{pred}$ (Eq. 2) (Omwange *et al.*, 2020), and the residual prediction deviation for prediction, (RPD_{pred}) (Forouzangohar *et al.*, 2008). RPD is defined as the ratio of the standard deviation of observed values ($STDEV_{obs}$) for all calibration samples to the $RMSE_{obs}$ (Eq. 3) for prediction samples. According to Baillères *et al.*, (2002); where a value above 2.0–3.0 can be used for rough screening.

$$R^2_{pred} = 1 - \frac{\sum_{i=1}^n (y_{pred} - y_{obs})^2}{\sum_{i=1}^n y_{pred} - y_{mean}^2} \quad (\text{Eq. 1})$$

$$RMSE_{pred} = \sqrt{\frac{1}{n} \sum_{i=1}^n (y_{pred} - y_{obs})^2} \quad (\text{Eq. 2})$$

$$RPD_{pred} = \frac{STDEV_{obs}}{RMSE_{pred}} \quad (\text{Eq. 3})$$

where n is the sample number, y_{obs} is the measured value, y_{pred} is the predicted value, y_{mean} is the average value.

Results and Discussion

Effects of chitin amendment on soil pH

The initial pH of soil samples was 5.5, 5.6, 6.0, 6.1, and 6.3 for control, fungal chitin 0.2%, fungal chitin 2.0%, sigma chitin 0.2% and sigma chitin 2.0%, respectively, as seen in Figure 2. The pH of the treated groups at day 0 was slightly higher than the control, mainly due to the neutral pH of the chitin materials. The pH of the control treatment was stable over the 122-day period, while that of the other treatment groups changed significantly over time with different tendencies during the entire experiment period. The pH of

the soil decreased gradually when a small amount of chitin was added; for instance, the pH of fungal chitin 0.2% and Sigma chitin 0.2% decreased consistently by 1.5 at the end of the experiment. However, the soil pH of the fungal chitin 2.0% and Sigma chitin 2.0% treatments increased to about 8.7 and 8.5, respectively, within 9 days after the treatment before they started to fall or remain stable for the remaining period. In general, addition of chitin had a significant effect on the soil pH; 0.2% chitin treatment slightly altered the pH of soils, making them more acidic; however, chitin containing more fungus biomass would have a long-lasting effect on the soil pH. At the end of the experiment (after 122 days), the pH for all the samples have become more acidic except for Fungal chitin 2.0% (Figure 2).

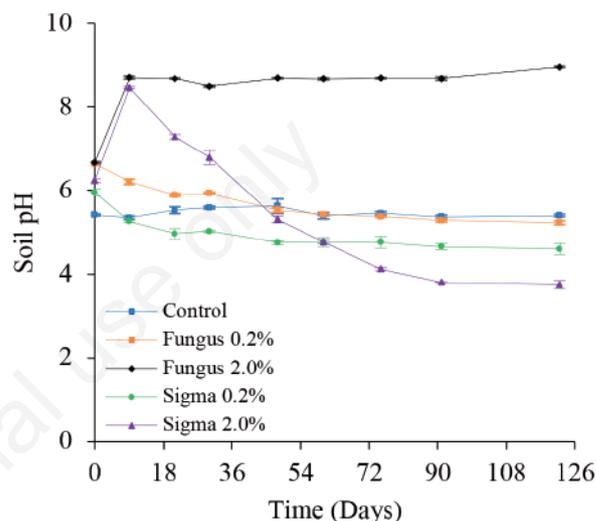


Figure 2. Soil pH changes over a period of 122 days after five chitin treatments (*i.e.*, 0.2% and 2.0% fungus chitin, 0.2% and 2.0% Sigma chitin, and the control or no chitin). The whiskers shown in the graph represent two standard deviations. Each value in the figure represents the average of three replicates.

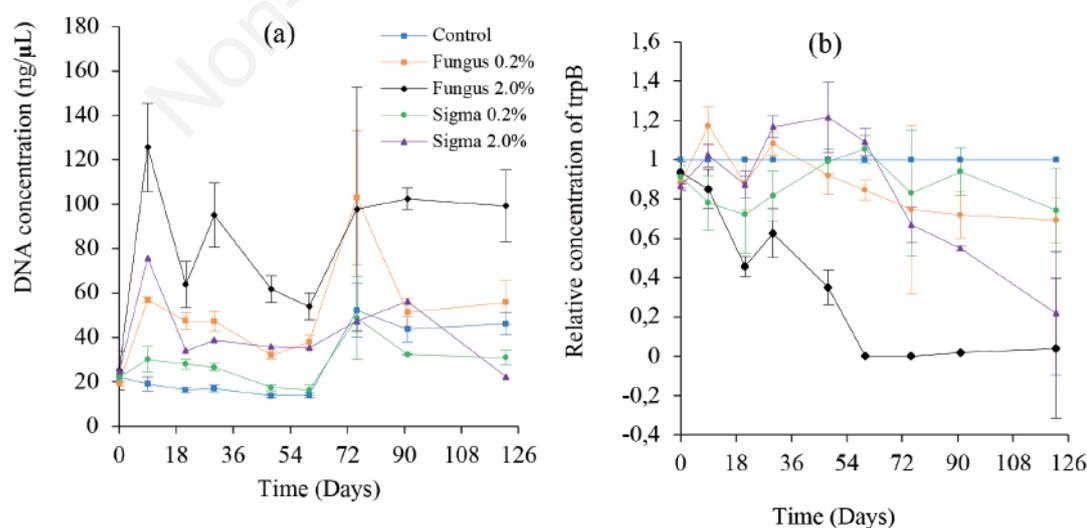


Figure 3. Soil DNA changes of the soil samples over a period of 122 days after five chitin treatments (*i.e.*, 0.2% and 2.0% fungus chitin, 0.2% and 2.0% Sigma chitin, and the control or no chitin): **a)** total DNA, and **b)** the *trpB* DNA concentration targeting on the identification of *Streptomyces scabies*. The whiskers shown in each graph represent two standard deviations. Each value in the figure represents the average of three replicates.

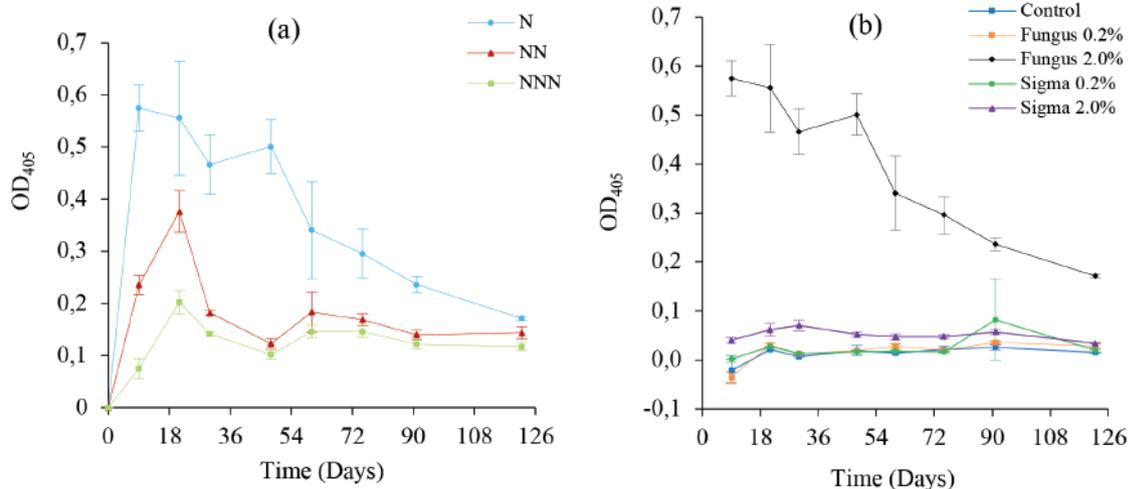


Figure 4. a) Soil chitinase activity for 2.0% fungal chitin treatment - all measurements at optical density at 405 nm (OD 405); N, β-N-acetylglucosaminidase activity; NN, chitinobiosidase activity; NNN, endochitinase activity detection. b) β-N-acetylglucosaminidase activity for all the treatments.

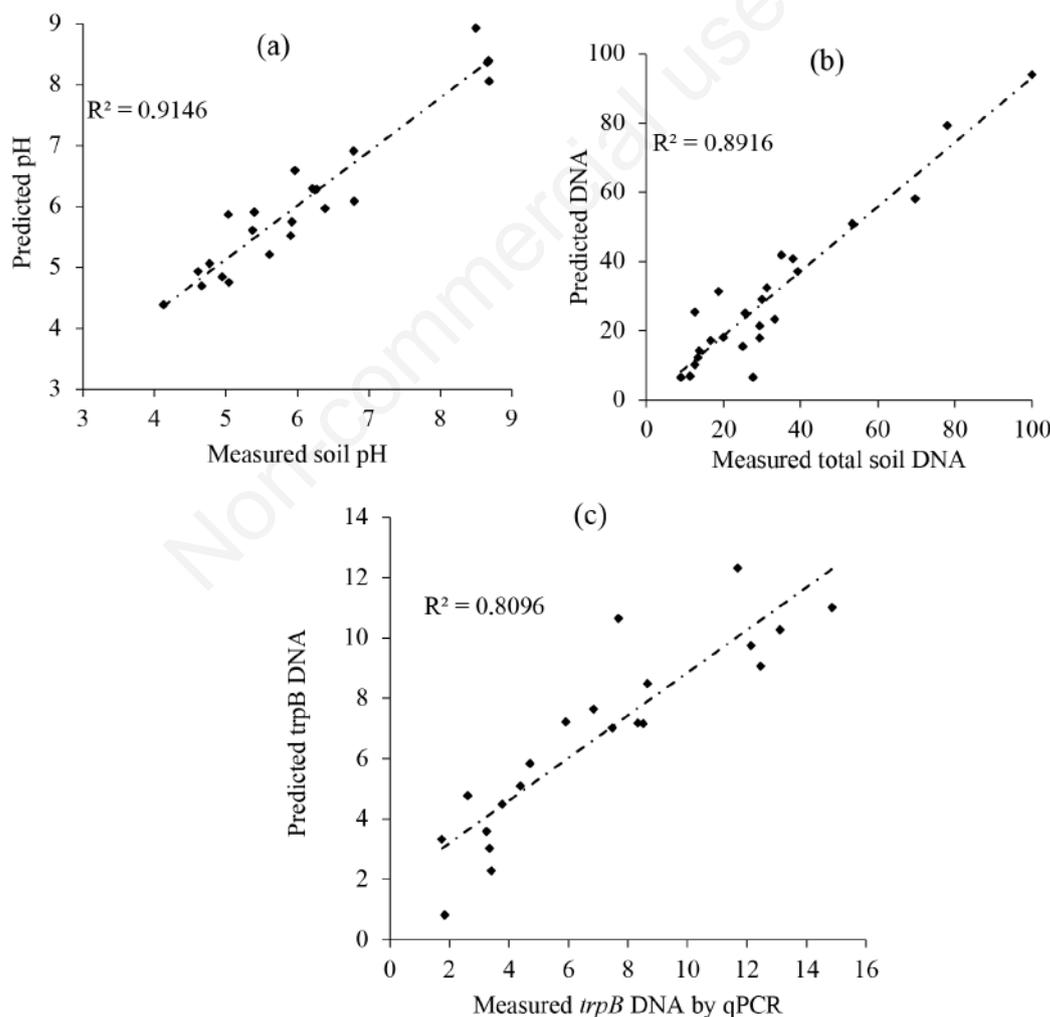


Figure 5. Partial least squares regression results for pH a), total soil DNA b) and *trpB* DNA c), using the second derivative of the reflectance spectra data for 900-1,660 nm.

Effects of chitin amendment on soil total DNA and the potato common scab pathogen DNA

The change in total DNA represented the modifications in the overall population of the microbiome. As can be seen from Figure 3a, total DNA for control samples was stable till day 60 and then increased, while the other treatments had higher total DNA and larger variations in the entire experiment period. The effect of fungus and sigma on total DNA was smaller after 90 days. At the end of the experiment, the fungus-treated soils had higher total DNA than the control samples, while the sigma chitin-treated soils had lower total DNA than the control samples. In Figure 3b, *trpB* DNA for control was unchanged, while *trpB* DNA varied significantly for other treatments. The *trpB* DNA significantly lower than the control after 60 days. For the fungus chitin 2.0% treatment, a significant increase in total DNA (about 5 folds of the initial amount) was observed; increasing amounts of total DNA in soils caused a decrease in relative DNA amounts of the pathogen, and this was confirmed by *trpB* DNA data (Figure 3b) as the *trpB* DNA was almost undetectable (Figure 3b). These results demonstrate that fungal biomass as a chitin-rich fertilizer can alter the soil's chemical and biochemical properties thereby impacting the pathogen population, and thus affecting crop productivity.

Chitinase activity

From the results presented in Figure 4, it can be noted that the highest chitinase activity was recorded in the 2.0% fungal chitin-treated soils, whereas there was no significant difference in the chitinase activity between the control and other treatments. Chitin is an integral component of fungal cell walls, but it is not present in plants. Most chitinase-producing bacteria have antagonistic effects on fungi, and most chitinase can also inhibit the growth of fungi *in vitro*. Therefore, chitinase is regarded as a safe and highly selective pesticide target, which has attracted a lot of research interest to study the application of chitinase in biological control (Herrera-Estrella and Chet, 1999; Chandrashekar *et al.*, 2012; Jabeen and Qazi, 2014). Three chitinase activities (N, NN, and NNN) peaked at 21 days and decreased as time progressed for fungus 2.0% treatment as seen in Figure 4a. Adding 2% of commercial Sigma chitin slightly increased the β -N-acetylglucosaminidase activity and no significant changes of chitinase activities were observed for low level of chitin treatments (Figure 4b). The data indicated that adding more fungal chitin could promote the generation of soil chitinases.

Regression analysis results

PLSR results from the soil reflectance spectra data for predicting pH, total DNA, and *trpB* DNA are shown in Table 2 and Figure

5. The PLS models with each of the three pre-processing methods (*i.e.*, second derivative, KM, and smoothing) could potentially monitor the pH, DNA, and *trpB* DNA of the soil. Among the three pre-processing methods, the second derivative had better results compared with the other two because of its ability to eliminate baseline noise due to the light scattering effect. For pH using the spectra data of the whole wavelength (900-1,685 nm), the highest R^2 (0.886) was achieved using the 2nd derivative model, and the highest RPD (2.640) was obtained from the smoothing model (Table 2). Since much noise was observed on the spectral data between 1,661-1,685 nm, PLS models for the spectral range between 900-1,660 nm have resulted in better predicted pH results with the R^2 values of 0.915, 0.864 and 0.887; RMSEP of 0.387, 0.651 and 0.573; and RPD of 3.476, 2.440 and 2.964 (Figure 5 a-c, respectively). While improvements in predicting the total DNA were also observed, improvements for *trpB* DNA were either small or nonexistent. The results for pH prediction using the spectral region of 900-1,660 nm suggest that NIR technique can be used for predicting the pH value of chitin treated soils; and the technique also has potential to classify chitin treated soils based on the level of total DNA. However, NIR technique is not likely to provide accurate assessment of *trpB* DNA in chitin treated soils. While the NIR technique showed promising results for assessing the three chemical/biochemical parameters, further studies are needed to test and evaluate the models using soil samples directly collected from the potato growing field treated with different levels of fungal biomass.

Contribution of individual wavelengths to PLS weights

Since the first LV generally has the highest weights on the PLSR model compared to the other LVs, it is thus helpful to understand how individual wavelengths contributed to this LV for the PLSR model. The model of the data (second order derivative, KM, and smoothing) for every parameter under consideration (pH, total DNA, and *trpB* DNA) are shown in Figure 6. Maximum peaks for the first LV was observed for pH at 1,428 nm over a broad wavelength range of 1,330-1,600 nm, with the cumulative contribution of 28.4% over the entire spectra; for DNA at 1,425 nm (a broad range of 1,330-1,600 nm) and a cumulative contribution of 25.3%. Both of these peaks had a positive contribution to the model (Figure 6 a,b). For *trpB* DNA (Figure 6b), 1,417 nm (a broad range of 1,350-1,555 nm) had a negative contribution and explained variance of 16.0%. Similar tendencies were observed for the model with the smooth pre-processing method. However, for the second order derivative method, more than one sharp peak emerged at 1,345 nm (positive contribution) 1,388 nm, and 1,428 nm (negative contribution) for pH; 1,329 nm and 1,355 nm (positive contri-

Table 2. Results from partial least squares regression models for the pH, total DNA, and *trpB* DNA of soil samples.

Wavelength range (nm)	Pre-processing method	pH				DNA (ng/ μ L)				<i>trpB</i> DNA (ng/ μ L)			
		LVs	R ²	RMSEP	RPD	LVs	R ²	RMSEP	RPD	LVs	R ²	RMSEP	RPD
900-1,685	2 nd derivative	7	0.886	0.633	2.466	8	0.903	14.256	2.424	7	0.805	1.899	2.173
	KM	8	0.826	0.734	2.211		0.811	14.792	2.324		0.566	2.752	1.442
	Smoothing		0.868	0.552	2.640		0.763	17.938	1.936		0.681	2.275	1.784
900-1,660	2 nd derivative	7	0.915	0.387	3.476		0.892	12.785	2.426		0.810	2.275	1.764
	KM	8	0.864	0.651	2.440		0.825	13.534	2.404		0.566	2.516	1.543
	Smoothing		0.887	0.573	2.964		0.796	17.425	2.174		0.662	2.316	1.728

LVs, number of latent variables; R², coefficient of determination for prediction; RMSEP, root mean squared error of prediction; RPD, ratio STDEV_{obs}-RMSE_{test}.

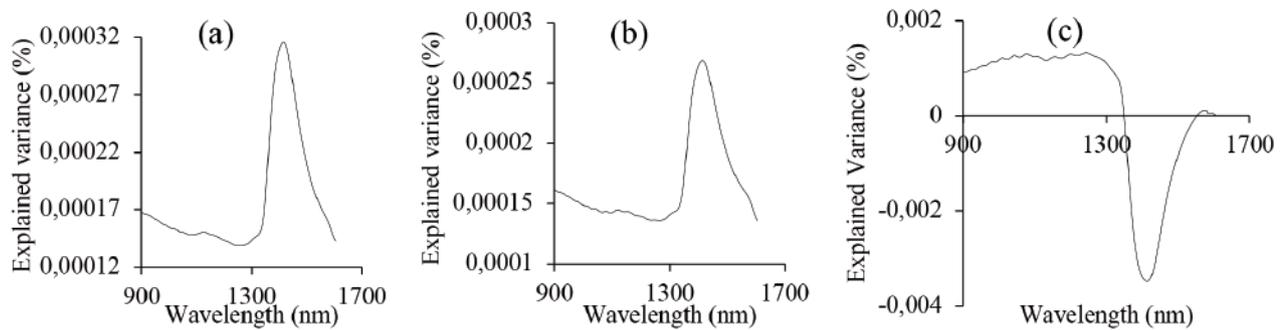


Figure 6. Representative weights for first latent variables (LVs) in the partial least squares regression models for pH **a**), total DNA **b**), and *trpB* DNA **c**), using the Kubelk-Munk pre-processing method.

bution) and 1,371 nm, 1,412 nm and 1,443 nm (negative contribution) for DNA and 1,340 nm (negative contribution) 1,379 nm and 1,419 nm (positive contribution) for *trpB* DNA respectively. Based on these findings, the wavelength range between 1,300-1,600 nm came out as the most significant wavelength region for the assessment of pH, DNA, and *trpB* DNA of chitin treated soil samples. It should be mentioned that water absorption occurs at the wavelength of around 1,450 nm, which is close to the peak wavelengths identified in this study. However, it should be mentioned that in the current study, the soil moisture content was maintained at around 50%, which has enabled us to minimize or eliminate the effect of moisture on the developed models and in the wavelength contribution analysis.

Conclusions

This research showed that chitin treatment has caused changes in the soil pH, total DNA, and *trpB* DNA. Small amounts of fungal chitin treatment (0.2%) slightly lowered the pH of the soil, making it more acidic; however, higher chitin treatments with more fungus biomass (2.0%) have resulted in a significantly higher soil pH over a longer period. This suggests that the fungal biomass of chitin-rich fertilizers can modify the chemical and biochemical soil properties, thus affecting the population of pathogens and eventually crop yields. The NIR reflectance technique, coupled with the PLSR modeling approach based on the 2nd derivative and smoothed data, was able to predict the three biochemical soil properties, i.e., pH, total DNA, and *trpB* DNA, with the best R^2 values of 0.915, 0.892 and 0.810, respectively. Further analyses of wavelength contributions showed that the spectral region of 1,300-1,600 nm was the most significant for predicting the soil biochemical properties. Hence, NIR reflectance spectroscopy could provide a fast and low-cost alternative method for assessing the change of biochemical properties of soil treated with fungal biomass. Finally, further studies are needed to test and evaluate the NIR models for assessing the biochemical properties of soil samples directly collected from potato growing fields.

References

Alcaraz de la Osa, R., Iparragirre, I., Ortiz, D., Saiz, J.M., 2020.

The extended Kubelka–Munk theory and its application to spectroscopy. *ChemTexts* 6:1-14.

- Andreo-Jimenez, B., Schilder, M.T., Nijhuis, E.H., te Beest, D.E., Bloem, J., Visser, J.H.M., et al. 2021. Chitin- and keratin-rich soil amendments suppress *Rhizoctonia solani* disease via changes to the soil microbial community. *Appl. Environ. Microbiol.* 87:e00318-21.
- Baig, M.A., Qamar, S., Ali, A.A., Ahmad, J., Qureshi, M.I., 2020. Heavy metal toxicity and tolerance in crop plants. In: Naeem, M., Ansari, A., Gill, S. (eds.) *Contaminants in Agriculture*. Cham, Springer, pp. 201-216.
- Baillères, H., Davrieux, F., Ham-Pichavant, F., 2002. Near infrared analysis as a tool for rapid screening of some major wood characteristics in a eucalyptus breeding program. *Ann. Forest Sci.* 59:479-490.
- Bautista-Baños, S., Hernández-Lauzardo, A.N., Velázquez-Del Valle, M.G., Hernández-López, M., Ait Barka, E., Bosquez-Molina, E., Wilson, C.L., 2006. Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. *Crop Prot.* 25:108-118.
- Blanco, M., Villarroya, I., 2002. NIR spectroscopy: A rapid-response analytical tool. *TrAC-Trend. Anal. Chem.* 21:240-250.
- Brady, N.C., Weil, R.R., 2008. *The nature and properties of soils*, 14th ed. Upper Saddle River, Pearson Prentice Hall.
- Cardenas, G., Cuellar, J.D., Casals, P., 1997. Chitin and tosil chitin applications as nematocides. *Bol. Soc. Chil. Quim.* 42:535-546.
- Chae, D.H., De Jin, R., Hwangbo, H., Kim, Y.W., Kim, Y.C., Park, R.D., et al., 2006. Control of late blight (*Phytophthora capsici*) in pepper plant with a compost containing multitude of chitinase-producing bacteria. *BioControl* 51:339-351.
- Chandrashekhara, K.N., Manivannan, S., Chandrashekhara, C., Chakravarthi, M., 2012. Biological control of plant disease management. In: Singh, V.K., Singh, Y., Singh, A. (eds.), *Eco-friendly Innovative Approaches in Plant Disease Management*. International Book Publishers and Distributors, Dehradun, chapter 10.
- Chang, C.-W., Laird, D.A., Mausbach, M.J., Hurburgh, C.R., 2001. Near-infrared reflectance spectroscopy-principal components regression analyses of soil properties. *Soil Sci. Soc. Am. J.* 65:480-490. d
- Chysirichote, T., Reiji, T., Asami, K., Ohtaguchi, K., 2014. Quantification of the glucosamine content in the filamentous

- fungus *Monascus ruber* cultured on solid surfaces. *J. Basic Microbiol.* 54:350-357. d
- El Hadrami, A., Adam, L.R., El Hadrami, I., Daayf, F., 2010. Chitosan in plant protection. *Mar. Drugs* 8:968-987.
- Forouzangohar, M., Kookana, R.S., Forrester, S.T., Smernik, R.J., Chittleborough, D.J., 2008. Midinfrared spectroscopy and chemometrics to predict diuron sorption coefficients in soils. *Environ. Sci. Technol.* 42:3283-3288.
- Ghehsareh, A.M., Samadi, N. 2012. Effect of soil acidification on growth indices and microelements uptake by greenhouse cucumber. *Afr. J. Agricultural Res.* 7:1659-1665.
- Herrera-Estrella, A., Chet, I., 1999. Chitinases in biological control. *EXS* 87:171-184.
- Hummel, J.W., Sudduth, K.A., Hollinger, S.E., 2001. Soil moisture and organic matter prediction of surface and subsurface soils using an NIR soil sensor. *Comput. Electron. Agr.* 32:149-165.
- Jabeen, F., Qazi, J.I., 2014. Potential of bacterial chitinases and exopolysaccharides for enhancing shelf life of food commodities at varying conditions. *Int. Res. J. Environ. Sci.* 3:87-93.
- Kokalis-Burelle, N., 2001. Chitin amendments for suppression of plant parasitic nematodes and fungal pathogens. *Phytopathology* 91:S168-S168.
- Krishnan, P., Alexander, J.D., Butler, B.J., Hummel, J.W., 1980. Reflectance technique for predicting soil organic matter. *Soil Sci. Soc. Am. J.* 44:1282-1285.
- Kumar, V., Singh, K., Shah, M.P., Singh, A.K., Kumar, A., Kumar, Y., 2021. Application of omics technologies for microbial community structure and function analysis in contaminated environment. In: Shah, M.P., Sarkar, A., Mandal, S. (eds.) *Wastewater treatment: cutting-edge molecular tools, techniques and applied aspects.* Amsterdam, Elsevier, pp. 1-40.
- Kumar, V., Thakur, I.S., Singh, A.K., Shah, M.P., 2020. Application of metagenomics in remediation of contaminated sites and environmental restoration. In: Shah, M.P., Rodriguez-Couto, S., Sevinç Şengör, S. (eds.) *Emerging technologies in environmental bioremediation.* Amsterdam, Elsevier, pp. 197-232.
- Liu, Y., Liao, W., Chen, S., 2008. Co-production of lactic acid and chitin using a pelletized filamentous fungus *Rhizopus oryzae* cultured on cull potatoes and glucose. *J. Appl. Microbiol.* 105:1521-1528.
- Nakatsu, C.H., Torsvik, V., Øvreås, L., 2000. Soil community analysis using DGGE of 16S rDNA polymerase chain reaction products. *Soil Sci. Soc. Am. J.* 64:1382-1388.
- Omwangi, K.A., Al Riza, D.F., Sen, N., Shiigi, T., Kuramoto, M., Ogawa, Y., Kondo, N., Suzuki, T., 2020. Fish freshness monitoring using UV-fluorescence imaging on Japanese dace (*Tribolodon hakonensis*) fish. *J. Food Eng.* 287:110111.
- Pearce, R.B., Ride, J.P., 1982. Chitin and related compounds as elicitors of the lignification response in wounded wheat leaves. *Physiol. Plant Pathol.* 20:119-123.
- Peng, X., Shi, T., Song, A., Chen, Y., Gao, W., 2014. Estimating soil organic carbon using VIS/NIR spectroscopy with SVM and SPA methods. *Remote Sens. (Basel)* 6:2699-2717.
- Poulsen, P.H.B., Møller, J., Magid, J., 2008. Determination of a relationship between chitinase activity and microbial diversity in chitin amended compost. *Bioresource Technol.* 99:4355-4359.
- Reddy, M.V.B., Arul, J., Angers, P., Couture, L., 1999. Chitosan treatment of wheat seeds induces resistance to *Fusarium graminearum* and improves seed quality. *J. Agr. Food Chem.* 47:1208-1216.
- Wang, Y., Huang, T., Liu, J., Lin, Z., Li, S., Wang, R., Ge, Y., 2015. Soil pH value, organic matter and macronutrients contents prediction using optical diffuse reflectance spectroscopy. *Comput. Electron. Agr.* 111:69-77.
- Xu, R., Falardeau, J., Avis, T.J., Tambong, J.T., 2016. HybProbes-based real-time PCR assay for specific identification of *Streptomyces scabies* and *Streptomyces europaeiscabiei*, the potato common scab pathogens. *Lett. Appl. Microbiol.* 62:153-159.
- Xuemei, L., Jianshe, L., 2014. Using short wave visible-near infrared reflectance spectroscopy to predict soil properties and content. *Spectrosc. Lett.* 47:729-739.
- Yin, H., Zhao, X., Du, Y., 2010. Oligochitosan: A plant diseases vaccine-A review. *Carbohydr. Polym.* 82:1-8.
- Zeng, D., Mei, X., Wu, J., 2010. Study and preparation of an environmentally friendly corn seed coating agent. *J. Plant Protect. Res.* 50:21-214.
- Zeng, D., Shi, Y., 2009. Preparation and application of a novel environmentally friendly organic seed coating for rice. *J. Sci. Food Agr.* 89:2181-2185.