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Plant-soil interactions and mulching affect the physico-chemical soil parameters and the physiological state of Solanum tuberosum L. and Phaseolus vulgaris L.

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Abstract. The soil physico-chemical characteristics, including the oxidation-reduction (redox) potential (ORP), pH (acid-base reactions), and temperature, are essential for all soil organisms, and in turn, influence fluctuations of these parameters within the soil substrate. This study aimed to compare the mutual effects of soil physico-chemical properties and plant performance under cultivation, with and without a soil covering (mulch) in interrow soil. The investigation was conducted over a complete growing season in an agricultural setup using two model crops - Solanum tuberosum L. (potato) and Phaseolus vulgaris L. (bush bean). Throughout our field work in a high-altitude region of Rhodope Mountains, ORP showed a negative correlation with both pH and temperature. We also observed an interdependence between ORP and plant growth. Specifically, ORP values tended to decrease compared to control setups without plants, depending on the plant species and their developmental stages. Mulching significantly reduced ORP, indicating enhanced redox processes driven by soil organism activity. In the presence of mulch, S. tuberosum displayed slight increases in leaf dimensions, chlorophyll content, and electron transport efficiency in the thylakoids, while the effects on P. vulgaris were much less pronounced. Overall, the data highlighted ORP as a sensitive indicator of soil state, with mulching improving both soil regeneration and plant performance. Our findings contribute to the understanding of plant-soil interactions, supporting sustainable agricultural practices.

Key words: crop physiology, plant-soil interactions, redox potential, regenerative agriculture, soil chemistry.

Introduction

More than 100 years ago the German soil scientist Lorentz Hiltner (1904) defined the rhizosphere as the soil volume around the root that is directly affected by the root's function. Since Hiltner's time, significant scientific effort has been devoted to understanding the mechanisms of the root-soil interactions within the rhizosphere. Soil physicochemical parameters, such as oxidation-reduction (redox) potential (ORP), pH, electrical conductivity, salinity, and temperature, have been experimentally shown to significantly impact the physiological state and nutrient uptake of plants, both in wild varieties and cultivated species (Husson, 2012).

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The Belgian researcher M. Pourbaix (Pourbaix, 1974) has derived a correlation between the redox potential and pH values of the rhizosphere system, identifying an optimal thermodynamic stability point of all nutrients essential for plant and microorganism growth. This relationship is particularly important in the context of the on-going climate change and its associated extreme weather events. Fluctuations in soil moisture, air content, and soil erosion may significantly alter the soil's physico-chemical balance, potentially making some nutrients unavailable to plants (Pais et al., 2023).

Soil parameters, as well as the plants themselves, directly influence a diverse array of soil borne microorganisms, including soil bacteria, saprophytic and mycorrhizal fungi, and protozoa (Bousset, 2019). In turn, plants and soil borne microorganisms have been shown to affect soil physico-chemical parameters (Molefe et al., 2023), within certain limits to maintain nutrient availability and flow in the rhizosphere (Williams et al., 2021). Plants exert this influence either directly, through acid-base active root exudates, or indirectly, by releasing exudates that promote the growth of specific microorganisms that, in turn, affect soil parameters and modulate plant mineral nutrition.

Optimal soil physico-chemical conditions positively influence plant development and vigor; in crops, these soil parameters also impact yield (Husson, 2012). Given the increasingly negative effects of intensive agricultural practices (Puissant et al., 2021), it is important to further understand root-soil-microorganism interactions and to develop practical tools for crop producers to utilize these mechanisms on their farms. Such tools should help mitigate the adverse effects of extreme weather on field crops, providing greater security for the farmers across diverse climate conditions.

To gain insight into the practical aspects of the rhizosphere root-soil interactions, this study was conducted under field conditions in the highmountain village Momchilovtsi, Smolyan region, Bulgaria. The research aimed to investigate the relationship between the variable soil physicochemical parameters - specifically soil ORP, pH and temperature - and the physiological performance of two model crops, Solanum tuberosum L. (potato) and Phaseolus vulgaris L. (bush bean). Additionally, we examined the effects of mulching (covering the soil) on these rhizosphere interactions, soil physico-chemical status, vegetative growth, and relative yield of the model plant species.

Materials and methods *Plant material*

Potato tubers (*Solanum tuberosum* L.): For our field experiments, we chose medium-late potato variety "Jelly", developed and patented by the German company Europlant Phlanzenzucht GmbH (https://www.europlant.biz/en/list-of-varieties/pdf/jelly/). This variety has low sensitivity to multiple diseases and it is well adapted to unfavorable conditions, making it suitable for organic farming. The potatoes were planted on June 15, 2023 and were harvested on November 8, 2023. Yield was estimated by measuring the total weight of the collected potatoes per growing area (72 m²) and is presented as a percentage relative to the control.

Bush beans (Phaseolus vulgaris L.): The bush bean variety we chose was "Elixir", developed and patented by the Dobrudzha Agricultural Institute, General Toshevo, Bulgaria. The variety is specifically adapted to be drought tolerant and shows resistance to several economically significant diseases (Genchev et al., 2011). It is highvielding variety and suitable for multi-phase harvesting. The bush beans were planted on June 15, 2023, and harvested in several phases: a single harvest of green beans for analysis on August 20, followed by three consecutive harvests of dried beans at 10-day intervals, with the final harvest on September 24, 2023, after which the beans vegetative cycle was completed. Yield was assessed by analyzing the weight of the bean pods, presented as a percentage relative to the control.

Experimental design

The experimental field is located near the village of Momchilovtsi in the Smolyan region of Bulgaria, with selected plots situated at 1400 m a.s.l. [41°39'32"N, 24°46'28"E]. Monthly precipitation data was obtained by the National Institute of Meteorology and Hydrology (Fig. 1A). The experimental setup was divided into two subplots, one for potatoes and one for beans (Fig. 1B and C), with each plot further divided into sections with and without mulch applied to the soil. We selected an equal growing area of 72 m² for all sub-plots. Additionally, two control sections – one mulched and one non-mulched –

were left without any model species or weeds growing on them in order to distinguish the effects of the living plants on the soil substrate.

As a type of mulch, we chose one of the most affordable and widely available options – wheat straw (Magdof & van Es, 2009; Iqbal et al., 2020). Two-year-old stored wheat straw was chosen to ensure that no commercial pesticide residues remained in the material. The mulch was applied on the designated bean and potato sub-sections following the initial hilling and weeding, conducted 31 days after crop planting. Wheat straw used as mulch is a versatile material – porous and lightweight when initially applied – making it prone to displacement by wind and compaction into a thin layer after rain, which allows good contact with the soil substrate. Over the season, a large portion of the straw decomposes, significantly reducing its weed suppressing effect due to the decrease in thickness. Therefore, based on these properties, we applied the straw mulch at least 20 cm in the rows and inter rows.



Fig. 1. Experimental design: (A) Monthly precipitation data for the experimental field (Source – National Institute of Meteorology and Hydrology, Bulgarian Academy of Sciences); (B) Potato model setup – mulched and non-mulched; (C) Bush beans model setup – mulched and non-mulched. (D) Rhizon sampler *in situ*.

Soil moisture samplers

The redox potential, pH and temperature of the soil solution were analyzed using soil moisture samplers (Rhizons) from the Dutch company Rhizosphere Research products BV. We used Type 10 Rhizon MOM samplers, which feature a 10 cm porous section and 12 cm tubing, with pore size 0.12-0.18 μ m. The Rhizons were installed in situ (Fig. 1D) within the root zone of the plants, and soil solution was extracted using a vacuum syringe to prevent atmospheric oxygen from diffusing into the sample, which could distort the results.

Soil parameters measurement

To analyze the soil moisture solution, we used the Milwaukee MW105 MAX Waterproof pH/ORP/Temp Portable Meter with a multipurpose electrode, offering up to 5-point pH calibration and automatic ORP calibration. The measurements were performed in the field immediately after extracting at least 10 ml of soil solution from the soil substrate. The manufacturer's instructions (https://milwaukeeinstruments.eu/media/d5/d9/82/1642147900/mw105_106.pdf) were followed during the analysis.

Soil composition

For soil texture and mineral composition analysis, four soil samples per sub-plot were taken at a depth of 20 cm, combined, and analyzed as a single representative sample to provide an overview of the entire test field. The mechanical composition and quantitative elemental analyses of the soil were conducted at the University of Forestry, Faculty of Forestry, Department of Dendrology according to Chaneva et al. (2022).

Plant physiological activity

The physiological vigor of the model species was assessed by measuring chlorophyll content and fluorescence using the OJIP-test method described in detail by Zhiponova et al. (2020), with a portable chlorophyl meter and Handy-PEA fluorimeter, Hansatech Instruments, UK. Morphometric analysis included the measurements of the area and dry weight of fully developed leaves according to Zhiponova et al. (2020).

Statistics

The ORP, pH and temperature data represent mean values of at least five measurements per time point. To evaluate statistical differences among variants, one-way ANOVA followed by the Holm-Sidak test was performed using Sigma Plot 11.0 software (p < 0.05). The same software was used for linear regression to demonstrate the relationship between ORP and both pH and temperature. The coefficient of determination (r) was calculated with a significance level p < 0.05. For plant leaf analyses, two consecutive biological repetitions were performed, each including a minimum of 20 individual plants. Statistically significant differences between non-mulched and mulched variants were determined by the Student t-test (p < 0.05).

Principal component analysis (PCA) of ORP, plant growth and yield parameters for nonmulched and mulched variants was conducted using the prcomp function from the stats package in R 4.4.1, with centering to zero and scaling to unit variance of the experimental variables. PCA graphs were plotted using the R package ggbiplot 0.55.

Results

Soil composition

An initial, representative soil analysis was conducted on the experimental fields to determine the soil composition, total amount of nutrients, soil organic matter and pH values at the time of planting the model species (Fig. 2). The analyses revealed sandy loam soil with a good overall amount of nutrient content and very high organic matter content, providing a strong foundation for supporting proper plant growth and maintaining soil physico-chemical activity.



Fig. 2. Soil composition analysis – (A) Mineral particles composition; (B) Total nutrient and organic matter content. Colour heat map indicates the increase from blue (minimal) to white (intermedial) and red (maximal).

Redox potential measurement of the soil solution

To fully assess the effect of the applied mulch, soil measurements were initiated 45 days after the mulch was applied onsite. Samples were taken at three consecutive time points from the control and bush beans fields (Aug to Sep) and at four consecutive time points from the potato fields (Aug to Oct). The final measurement for the bean plot was on day 101 after sowing (September 24, 2023), coinciding with the last harvest of the dried beans. The final measurement for the potato field was conducted on day 127 after planting. At these later stages of development, both crops had entered their senescent phase, during which they exhibited minimal metabolic activity.

The soil ORP provides insight into the dynamics of oxidation-reduction reactions (Husson, 2012). An excess of electrons in the system correlates with negative ORP values in the analyzed soil moisture sample, indicating a more reducing system capable of electron exchange. The ORP method proved high sensitivity to mulching, as the effect was detected as early as the first time point in August, when all the mulched setups showed a decreased ORP compared to the nonmulched ones (Fig. 3). A distinct difference in the ORP profiles between the control and potato fields was observed (Fig. 3A, B). The ORP values in the potato field were statistically lower than those in the control, even at the final potato measurement when the plants were dry and nearing harvest.

A similar trend was observed in the bean fields, where a steady decrease in the ORP was recorded. However, unlike the potato field, the differences between mulched and non-mulched plants were more pronounced in the bean field (Fig. 3C). A sharp spike in ORP was recorded during the final measurement of the bush bean plots when the beans had already been harvested, and the plants were dry.

In conclusion, the presence of mulch and plants acted as a natural canopy leading to an overall decrease in ORP. In the absence of cover, either due to lack of mulch or plant drying, ORP values increased.

pH and temperature of the soil moisture solution measurements

In the current set of experiments, no statistically significant difference in pH values was observed between the mulched and non-mulched setups one month after mulching (Aug, Table 1A).

In the second month, the pH of the soil without plants began to drop, which also occurred in the soil without mulch (Sep, Table 1A). This effect was observed in *P. vulgaris*, likely due to plant senescence at this stage.

In *S. tuberosum*, which was harvested at the beginning of November, the pH generally remained constant, with a decrease only observed at the beginning of September (2 Sep, Table 1A).

The pH data were consistent with the soil temperature data, showing the same trend (Table 1B). The lower temperature at the beginning of September reflects a sudden drop in environmental temperature, which seems to impact the pH (Table 1A) and the ORP (Fig. 3). For *S. tuberosum*, the lower temperature in October did not affect the pH and ORP, which may be linked to the advanced developmental stage and maturation of the potato tubers.

Relationship between soil parameters – ORP, pH, and temperature

To examine the relationship between soil ORP, pH, and temperature, we performed a linear regression analysis between each pair of parameters (Table 2).

The ORP showed a negative correlation with both pH and temperature, with a stronger relationship established between ORP and pH.

A comparison between non-mulched and mulched setups revealed a stronger interdependdence among the three soil parameters in the presence of mulch.



Fig. 3. Soil oxidation reduction potential (ORP) analyses. Measurements were performed on soil with non-mulched and mulched plant variants and control without plants (from Aug to Oct).
(A) Soil without plants; (B) Soil with *S. tuberosum*; (C). Soil with *P. vulgaris*. The data are presented as mean ± SE (n ≥ 5). A one–way ANOVA (Holm–Sidak) test was applied to determine statistical differences between the variants, which are denoted by different letters.

Table 1. Soil pH and temperature. A comparison of the soil pH values (A) and measured soil solution temperature (B) between the potato and the bush bean fields with and without mulch and the control fields with bare soil and mulch on top of the soil, but no plants. Values' increase is shown as a colour heat map from blue (minimum) to red (maximum). The data are presented as mean ($n \ge 5$). A one–way ANOVA (Holm–Sidak) test was applied to determine statistical differences between the variants, which are denoted by different letters.

А					
	рН	20 Aug	2 Sep	24 Sep	23 Oct
	soil without mulch	7.0ª	6.9 ^a	5.5 ^d	-
	soil with mulch	7.2ª	6.9 ^a	6.1 ^b	-
	S. tuberosum non-mulched	7.2ª	7.0ª	7.2 ^a	7.2ª
	S. tuberosum mulched	7.3ª	6.5 ^b	7.2 ^a	7.2ª
	P. vulgaris non-mulched	7.1ª	6.9ª	6.7 ^{ab}	-
	P. vulgaris mulched	7.1ª	7.1ª	6.7 ^{ab}	-
В					
	T°C	20 Aug	2 Sep	24 Sep	23 Oct
	soil without mulch	26.1ª	20.1c	19 ^d	-
	soil with mulch	26.9ª	21.6 ^c	19 ^d	-
	S. tuberosum non-mulched	25.7ª	25.7ª	24.1 ^b	20.9 ^c
	S. tuberosum mulched	25.6ª	21.3c	25.7ª	20.1c
	P. vulgaris non-mulched	25.7ª	19.9 ^d	18.8 ^d	-
	P. vulgaris mulched	27.0ª	22.3 ^b	21.4 ^c	-

Table 2. Relationship between pH and temperature with ORP by linear regression. The respective coefficient of correlation (r) and significance P < 0.05 (n = 9) are shown. The negative sign in front of r stands for inversely proportional dependence.

		non-mulched		mulched	
y	x	r	Р	r	Р
ORP	pН	-0.888	< 0.01	-0.957	< 0.001
ORP	Т	-0.668	0.049	-0.757	0.018
pН	Т	0.625	0.072	0.851	< 0.01

Physiological vigor of the model plants

The physiological state of the plants was evaluated by the sensitive chlorophyll fluorescence approach (Fig. 4A). An increase in the quantum yields of primary photochemistry and electron transport of photosynthesis (ϕ Po and ϕ Eo) was observed in the mulched potato leaves, leading to much higher photosystem II productivity (PIABS) and both photosystems' performance (PITOTAL). The absorbed photon flux per reaction center (ABS/RC) decreased in potatoes while it increased in beans, with this being the only statistically significant difference between the experimental variants for the latter. Mulching did not affect the number of active reaction centers per cross section (RC/CSO) nor the quantum yield of reduction of end photosynthetic electron acceptors (ϕ Ro).

In line with the enhanced electron transport in photosystem II and between photosystems II and I, the chlorophyll content, leaf size and biomass were increased in the mulched potatoes. In the bush bean, the physiological parameters measured at this developmental stage did not show any significant dependence on the presence of mulch, except for a slight increase in chlorophyll (Fig. 4 B-D).

Throughout the vegetative cycle of the model species, no substantial pest or disease invasion was observed. In the potato fields, sporadic occurrences of Colorado beetles (*Leptinotarsa decemlinea-ta*) were encountered, but their population did not reach economically significant levels, despite no pest control measures being applied to the crops.

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Fig. 4. Physiological analyses. Measurements were performed on *S. tuberosum* and *P. vulgaris* nonmulched and 3-week-mulched variants (on 20 Aug). (A) Chlorophyll fluorescence parameters comparison profile; (B) Chlorophyll content; (C) Leaf area; (D) Leaf DW. The asterisks show a significant difference between non-mulched and mulched plant variants (Student *t*-test, *p* < 0.05)

Yields

The experimental setup was completed with the measurement of the harvested yields. Each sub-field represented a growing area of 72 m². Due to the difference in growth patterns between the two model crops, the harvests were carried out in multiple phases for the bush bean until September, and once in November for the potatoes. The analysis of the bean pod weight in the mulched and non-mulched setups did not show any statistically significant difference (Fig. 5B).

Due to the multi-phase harvest of the dried beans and some losses from dry pod openings, it was not possible to measure accurate values for the total dry bean yields of the two setups. The potato yield was measured exactly for both setups (Fig. 5A). In the potato experimental field, a significant yield difference of 16 kg (i.e. 7%) was observed between the two setups.



Fig. 5. Harvest. (A) Total potato yield per growing area; (B) Pod weight of bush beans.

Relationship between soil physico-chemical parameters and plant physiology

To estimate the effect of mulching on the physico-chemical soil properties, a PCA analysis was performed (Fig. 6). The data highlighted that the presence of mulch contributed to the plant growth parameters and yield. As shown in Tables 1 and 2, mulching was correlated with lower ORP and increased pH, while the temperature showed less interdependence.

To summarize the differences between the experimental variants principal component analysis (PCA) based on the parameters considered in the study was performed (Fig. 6). The first and second principal components (PC1 and PC2) together explained 86.2% of the total variance between the groups. Soil pH was the variable that primarily contributed to PC1. Leaf area and biomass (DW), as well as soil ORP, were the other major contributors to PC1, while soil temperature

(T) had a minor contribution. PItotal and PIABS mainly determined PC2. Chlorophyll content (Chl) and yield contributed almost equally to both PCs. PC1 increased with all parameters except pH, yield and Chl, while PC2 increased only with ORP. Mulched plants were characterized with lower PC1 and PC2 values compared to non-mulched for both species.

However, the difference was much larger for *S. tuberosum* than for *P. vulgare*, especially in PC2. Mulching was associated with an increase in soil pH, plant Chl content, photosynthetic performance, and yield, as well as a decrease in soil ORP and temperature. On the other hand, the main disparity between the model species was along the PC1 axis, caused by wider deviations in pH, ORP and leaf morphology (size and weight). The indicates that *P. vulgaris* grew in more acidic and oxidizing soil than *S. tuberosum*, regardless of mulching.



Fig. 6. Relationship between soil parameters and plant performance. PCA of: Soil ORP; pH; Temperature (T); Leaf area and DW; Chlorophyll (Chl) content; Photosynthetic performance indexes (PI_{ABS}, PI_{TOTAL}); Yield.

The state of the mulch

A visual observation of the changes in the applied mulch was also carried out throughout the vegetative life cycle of the plant species (Fig. 7).

To monitor the weed suppressing effect of the mulch, no weed management was applied to the test fields. At the beginning of the experiment, the mulch layer was approximately 20 cm thick. As the year progressed, some areas of the mulch layer were decomposed due to microbiological activity, indicated by the presence of fungal fruiting bodies, including species such as Peziza sp. and Coprinus sp., observed after each rain event. By the end of the growing season, as the plants dried out, a thick volunteer wheat undergrowth, originating from the straw used as mulch, appeared on the bean field (Fig. 7B). On the potato field, no wheat growth was observed, likely due to the dense potato foliage shading the wheat seeds for an extended period (Fig. 7A). To avoid any potential effects from the weed species and volunteer wheat plants, which grew mainly in the interrows, soil samplers were always inserted into the central root zone of the model plants, focusing on plants that had no weeds growing in the vicinity of their root zone.

Discussion

The accumulated data shows a correlation between the existence of a living plant and the state of the soil's physico-chemical parameters. The control sections, with no plants, provide results based on the existing physico-chemical properties of the initial soil substrate, the atmospheric conditions and the living microbiota inhabiting that substrate. The potato and bean fields were exposed to the same atmospheric conditions and similar soil substrate, but unlike the controls these sub-plots had the living model plant species growing. We assume that the presence of living model species modified the soil microbiota (Molefe et al., 2023), promoting microbiological groups associated with the root zone of the plants. Further in-depth taxonomic identification is required to reveal the present soil microorganisms. The clear decrease in ORP values in the potato and bean fields, compared to the controls confirms that the presence of a physiologically active plant can significantly affect the physico-chemical state of the soil solution. This is further emphasized by the final ORP measurements in both the potato and bean plots, where the plants were in the final stages of their senescent phase and no longer physiologically active. In these conditions, the measured ORP values were comparable to those in the controls for the bean plot and they were lowest for the potato plots, in both mulched and non-mulched setups. It could be hypothesized that the potato plants keep the soil moisture, which is beneficial for the soil microbiome and would be supported by biodiversity analyses (Hemkemeyer et al., 2024).



Fig. 7. The experimental fields at the end of the growing period. (A) The condition of the field and the mulch on the potato field; (B) The condition of the field and the mulch on the bush bean field.

The effect of the mulch on lowering ORP values persisted across all setups – beans, potatoes, and controls. Since the mulch helps reduce soil moisture evaporation (Ji et al., 2001) and reduced the impact of the UV solar radiation on the soil and soil microorganisms, it is logical to conclude that the positive effect is due to the increased population of soil flora and fauna in the favorable growing conditions under the mulch cover. The reduced water evaporation could explain the higher yields in the mulched potato field compared to the non-mulched field. This could also account for the slight increase in the physiological activity of the mulched plants over

the non-mulched plants. Although no qualitative or quantitative data were obtained on soil flora and fauna, a potential increase in soil borne microorganisms, in the mulched setup may have contributed to the improved crop yields (Hemkemeyer et al., 2024).

Interestingly, at the third ORP measurement of the potato crop, at the end of September – when the plant was at its peak physiological strength the difference between the mulched and nonmulched setups was the smallest. In contrast, a 9 mV difference in ORP was observed between the two setups during the peak growth period of the bush beans in August and September. This is

likely due to the differing growth habitus of the two species. During full growth, potatoes form a thick canopy of leaves that create a dense cover, mimicking the effect of mulch by shading the soil. Bean plants, however, form smaller, less dense bushes that do not completely cover the inter rows, leaving the non-mulched soil exposed to drought-induced oxidation (Hu et al., 2012), which results in higher, positive ORP values compared to the mulched plants.

The state of the fields after harvest will continue to be monitored, as it would be important to investigate whether the emerging volunteer wheat plants will affect the soil's physicochemical parameters after the main crop has been removed. If this is the case, the newly germinated wheat could play a beneficial role (Vagnerova et al., 1960) in maintaining soil borne flora and fauna in the field for a longer period within the growing season.

Conclusions

The current setup is the first in Bulgaria to attempt to measure both qualitatively and quantitatively the root-soil interactions of potato and bean plants in a field setting. The accumulated statistical data showed that the model crops actively influence the soil substrate parameters. Our research highlighted the oxidation-reduction (redox) potential (ORP) as the most sensitive parameter which can be applied to estimate the impact of agricultural activity.

Furthermore, it was demonstrated that the practice of straw mulching could be of great importance for farmers, especially in regions with extended drought periods, as it helps maintain root-soil interactions for a longer period. Despite occasional variations in the measured results, the experimental setups using straw mulch as soil cover showed a reduction in the redox potential values compared to the setup with no mulch. Throughout the growing season there was a visible correlation between the plant growth stage and the redox potential values in the soil substrate the plants occupy. This indicates that the crops actively affect the physico-chemical properties of the soil substrate.

This study serves as a foundation for in-depth fundamental and applied research required to identify the mechanisms of plant-soil interactions under specific soil regions, plant species, and environmental conditions. The knowledge gained would contribute to sustainable agriculture by promoting efficient soil preservation and increased plant productivity.

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