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SOIL FUNGI DIVERSITY AND POPULATION BASED ON THE SOIL SUBTYPE IN INTENSIVE APPLE ORCHARDS OF CENTRAL RUSSIA

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SUMMARY

An investigation of the population and genus composition of soil fungi in the rhizosphere of intensive 12-year-old apple orchards grown on the three distinct chernozem soil subtypes—podzolized, leached, and typical—inspired this study. Research transpired in the growing seasons of 2024–2025 across the Tambov and Lipetsk regions, Central Russia. Soil samples' collection had three different depths (0–10, 10–30, and 30–60 cm). From 12 genera, fungi isolation primarily included *Penicillium*, *Mucor*, *Aspergillus*, *Fusarium*, *Botrytis*, *Trichoderma*, *Rhizopus*, and *Verticillium,* with data on *Penicillium*, *Mucor*, *Aspergillus*, *Verticillium,* and *Pythium* presented graphically. Meanwhile, the remaining genera presentations were in the narrative form. The podzolized chernozem soil subtype, characterized by the highest acidity and humus content, yielded a higher number and diversity of fungal genera, in which the *Botrytis* and *Verticillium* were predominant. Conversely, *Aspergillus* was the characteristic of the leached chernozem, while *Mucor* and *Rhizopus* dominated the typical chernozem. A considerable positive correlation ($r > 0.85$) was evident between the soil's humus content and acidity (low pH) and the total abundance of the investigated fungal genera. The results highlighted the crucial role of the chernozem soil subtype, governed by its specific chemical parameters, in structuring the fungal community in the apple tree rhizosphere.

Keywords: Genera of soil fungi, chernozem soil subtype, apple tree, intensive orchards, apple tree rhizosphere, soil microbiology, Czapek's medium

Key findings: The results showed the diversity and abundance of fungal genera in the apple tree rhizosphere largely depend on the soil pH and humus content, which generally materialize in differences in the subtypes of chernozem.

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INTRODUCTION

Currently, more than 80,000 species of fungi are common; however, only 17% of these have received successful studies (Bridge and Spooner*,* 2001). Soil fungi represent a crucial group of microorganisms, actively participating and playing a remarkable role in environmental quality improvement and nutrient supply to crop plants. Therefore, the precise identification and exploration of local soil fungal species are essential for compiling comprehensive fungal checklists (Kichu *et al.,* 2019).

Soil fungi play multifaceted roles in plant ecology and boosting sustainable agriculture. For instance, mycorrhizal symbiotic associations are vital for plant nutrition and stress resistance, especially in arid arable regions. The fungal positive effects have been apparent in plants like *Aristida pungens*, *Retama raetam*, and *Astragalus armatus* in the Algerian steppes (Amina and Hamida, 2025). Among the soil fungi, many are pathogenic and conditionally pathogenic for apple trees: *Penicillium*, *Cladosporium*, *Fusarium*, *Mucor*, *Trichotecium*, and *Alternaria* (Kuzin *et al.,* 2025).

Furthermore, specific soil fungi are also useful for biological control. Fungi, such as *Penicillium simplicissimum*, *Clonostachys rosea*, and *Purpureocillium lilacinum,* can be applicable as biological control agents against wax moth larvae and potentially other insect pests (Pravallika *et al.,* 2023). Conversely, certain phytopathogenic fungi, including *Penicillium citrinum*, *Bipolaris sorokiniana*, and *Alternaria alternata*, emerged to be common in the rhizosphere of various fruit and medicinal plants such as white mulberry (*Morus alba*) and Melia (*M. azedarach*, Chinaberry). Combating *Alternaria alternata* led to the identification of the antagonist soil fungus *Purpureocillium sodanum* as an alternative to standard *Trichoderma* strains (Kaur and Kaur, 2025). The rhizosphere of the medicinal plants, such as *Eclipta prostrata* and *Mentha arvensis* frequently had arbuscular mycorrhizal fungi dominating them, specifically *Acaulospora* and *Glomus* (Songachan *et al.,* 2023).

Fungal communities enunciate high geographic and environmental specificity. For instance, in the soils of Northeastern India, the predominant fungal genera include *Absidia, Alternaria, Aspergillus, Cladosporium, Fusarium, Geotrichum, Mortierella, Mucor, Penicillium*, and *Trichoderma* (Kichu *et al.,* 2019). In the same region, *Aspergillus* was notably the dominant genus in banana plantations in Nagaland soils (Temjen *et al.,* 2022). *Penicillium janthinellum* and *Trichoderma spirale* appeared to be common in Brazilian forest soils (Rodrigues *et al.,* 2014), while *Fusarium* (over 10 species) has reached reports of highest diversity in Dutch soils (Crous *et al.,* 2021). Keratinophilic fungi, such as *Chrysosporium keratinophilum* and *Microsporum gypseum,* predominate in the arable soils of Tunisia (Anane *et al.,* 2015).

In addition to fungi's potential role in symbiosis and biocontrol, fungi considerably contribute to soil chemistry. *Aspergillus niger* (strains AD-A2 and AG-B1) and *Trichoderma hamatum* (strain T-113) have been noticeable in better dissolving the phosphates and releasing organic acids (octanoic, acetic, and crotonic) and complex compounds (esters and methylacetone) into the soil (Al-Zubaidi and Al-Taie*,* 2022). Moreover, establishing the stimulating effect of the fungi *Trichoderma harzianum* and *Trichoderma aureoviride* on root and shoot growth and development of rice has succeeded in West Bengal, India (Sarkar *et al.,* 2022).

In the context of perennial crop plants, the fungal community of the fruit tree rhizosphere was successful in recognizing it as highly dependent on the species composition of both weeds and cultivated vegetation in different orchards (Deakin *et al.,* 2018). Past studies revealed the abundance of beneficial fungi like *Scutellinia*, *Penicillium*, *Lecythophora*, and *Paecilomyces* in the soil showed a positive correlation with M9 apple rootstock growth. Conversely, increased abundance of phytopathogenic genera, such as *Acremonium*, *Fusarium*, and *Cylindrocarpon,* severely inhibits plant growth (Franke-Whittle *et al.,* 2015). Therefore, the management of pathogenic fungi can be achievable through biological methods—through inoculation of the apple tree rhizosphere with the arbuscular mycorrhizal fungus *Paraglomus sp*., which significantly reduced the abundance of harmful *Fusarium* species and accelerated the growth of apple trees (Wang *et al.,* 2024).

Furthermore, environmental factors can also alter the community structure of microorganisms, as gamma radiation in an apple orchard soil on the Loess Plateau of Northern China revealed to change the species composition of *Fusarium* fungi (Caputo *et al.,* 2015). As a result of the accumulation of plant polyphenols in soils, the structure of the microbial community changes and soil fatigue occurs (Arafat *et al.,* 2020). In humid regions of China, the research indicates that genera such as *Gibberella*, *Fusarium*, and *Cryptococcus* accumulate in older apple orchards, posing a threat to trees (Xu *et al.,* 2022). Pathogenic fungi *Rhizoctonia solani, Pythium ultimum, Phytophthora cactorum, Fusarium oxysporum,* and *Dematophora necatrix* have prevailed in the soils of old, decaying orchards, and along with bacteria and actinomycetes, were more abundant than in the soil of young orchards (Singh *et al.,* 2020). Given the significant influence of fungal communities on the health and productivity of fruit crops, the knowledge about the specific composition of fungal genera regarding local soil subtypes—such as the various chernozems in the Russian Federation—is crucial for developing targeted management strategies in intensive apple production. The aim of the study was to determine the abundance of the 12 most common fungal genera in three subtypes of chernozem in the rhizosphere of 12-year-old apple trees and identify soil factors affecting the abundance of these fungi.

MATERIALS AND METHODS

Study area and experimental design

The research began during the growing seasons of 2024–2025 in intensive 12-year-old apple orchards located in the Tambov and Lipetsk regions of the Russian Federation. The study utilized three distinct chernozem soil subtypes from different agricultural enterprises. These are podzolized chernozem: Timiryazevsky Agricultural Research Center (Lobo apple tree variety, plot coordinates 52.339364, 38.308887); typical chernozem: Dubovoye JSC (Lobo apple tree variety, plot coordinates 52.596457, 40.288994); and leached chernozem: Agrofirma named after 15 Let Oktyabrya CJSC (Lobo apple tree variety, plot coordinates 52.979927, 38.996625). The soils under the studied orchards entailed identification according to the Classification and Diagnostics of the Soils of the USSR (1977) and the World Reference Base for Soil Resources (WRB) (IUSS Working Group WRB, 2022). All the orchards featured 62–396 rootstocks, with a tree layout of 4.5 m × 2 m. The inter-row areas of the orchards received maintenance under black fallow, using the IRRI-GO drip irrigation system in a compensated tube with a diameter of 16 mm. The distance between the emitters (droppers) is 0.5 m; the tube wall thickness is 44 Mil. One emitter pours 1.6 l in 1 h. Watering ensures optimal soil moisture for apple trees of 80% of the maximum field moisture capacity of the soil. The soils had characteristics of a granulometric composition, ranging from medium to heavy loam.

Soil sampling and agrochemical analysis

Soil samples collected came from the tree-row strips (rhizosphere area) at three specific depths: 0–10, 10–30, and 30–60 cm. Three soil samples taken from each soil layer comprised nine soil samples coming from each soil subtype. Agrochemical analysis proceeded according to standard regional instructions: humus content determination used Tyurin’s method (State Standard 26213-2021); hydrolytic (total) acidity detection was in accordance with State Standard 26212-91. Exchange acidity (pH_{KCL}) succeeded its determination by the ionometric method (State Standard 26483-85).

Microbiological analysis

After sampling, the transport of soil samples on the same day in closed glass boxes continued to the laboratory, where their storage for no more than a day in the refrigerator had a temperature of +4 °C. The soil did not undergo drying. The soil fungi population determination used the dilution plate technique. Fungi culturing in Petri dishes utilized Czapek's medium. Aqueous soil suspensions' plating was at the following sequential dilutions: 10^{-2}, 10^{-4}, and 10^{-6}. For the study, taking 1 g of soil proceeded in an aqueous suspension preparation with a dilution of 1/100, 1/10000, and 1/1000000 (the initial dilution is 1 ml of suspension per 10 ml of sterile water). After the required dilution, adding 1 ml of the finished suspension to the Petri dish followed. After exposure in a thermostat at a temperature of 20 °C–25 °C, colonies incurred identification by their color, size, shape, nature of mycelium, and sporulation using a Micmed-5 microscope (Methods of Microbiological Soil Control, 2004). The analysis was morphological. After the first 2–3 days of incubation in the thermostat, the nature of the mycelium entailed determination, and in the next seven days, detecting the nature of sporulation ensued. The identification of fungi took place only at the genus level.

Statistical analysis

The study carried out the mathematical processing of the digital material by the method of dispersion analysis (Dospekhov, 2011). The expression of results was the mean ± standard deviation for XXX repetitions. Linear correlation analysis, as performed, utilized the Microsoft Excel software, with the statistical significance evaluated by the Student's t-criterion. The smallest significant difference was 20 CFU/g (Figures 1 to 5); the accuracy of the experiment ranged (P) from 3% to 5%.

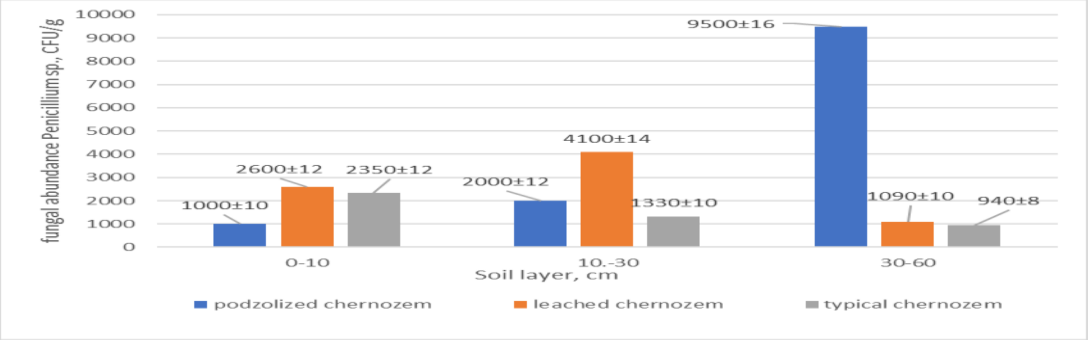

Figure 1. Abundance of *Penicillium* spp. fungi in the rhizosphere of apple trees in intensive orchards, depending on the soil subtype (CFU/g).

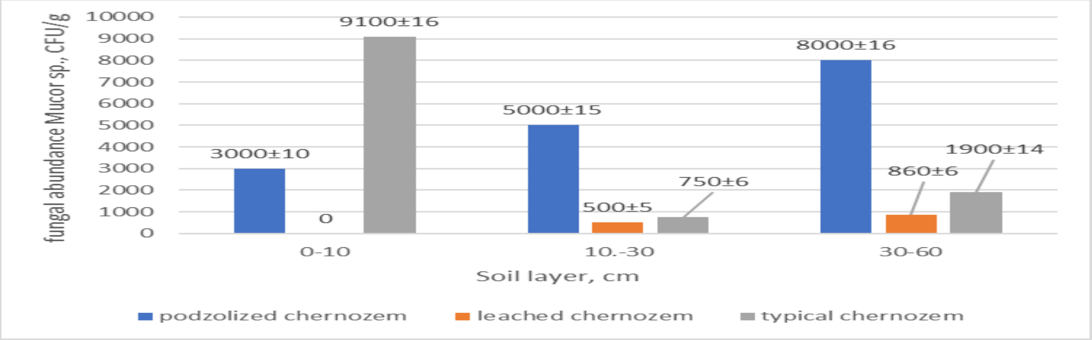

Figure 2. Abundance of fungi *Mucor* spp. in the rhizosphere of apple trees in intensive orchards, depending on the soil subtype (CFU/g).

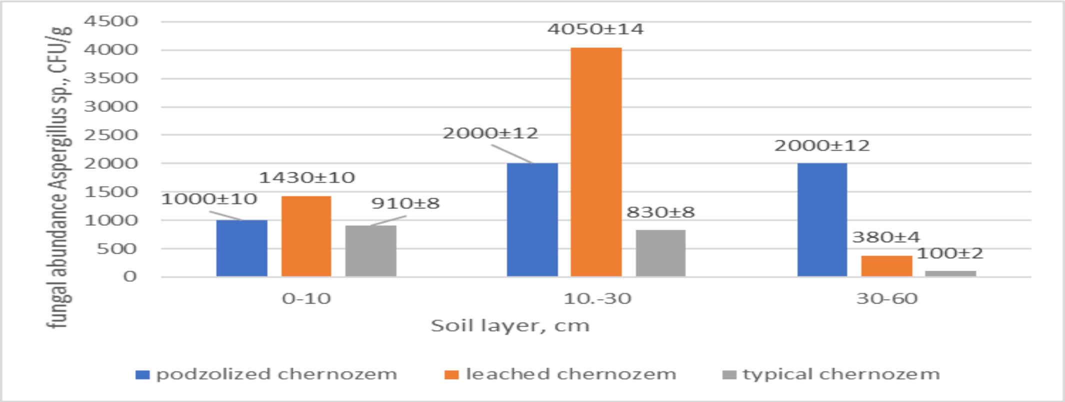

Figure 3. Abundance of *Aspergillus* spp. fungi in the rhizosphere of apple trees in intensive orchards, depending on the soil subtype (CFU/g).

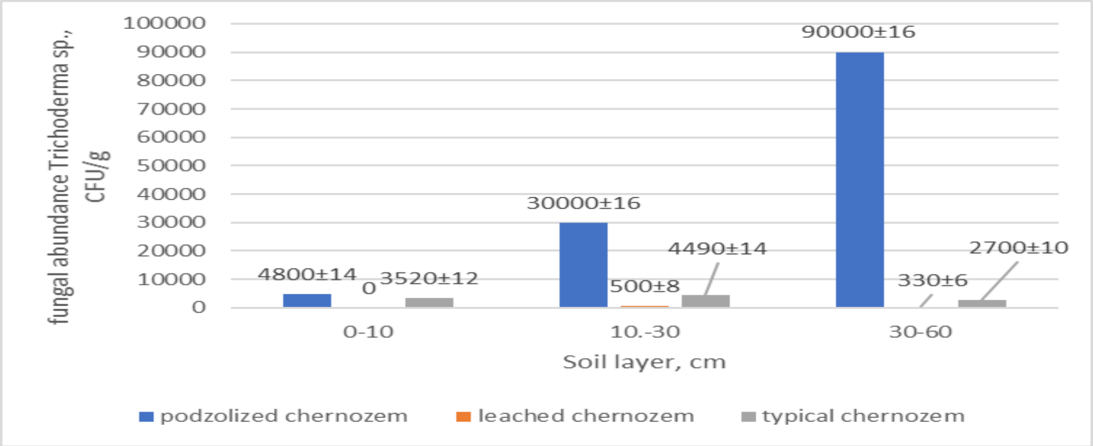

Figure 4. Abundance of *Verticillium* spp. fungi in the rhizosphere of apple trees in intensive orchards, depending on the soil subtype (CFU/g).

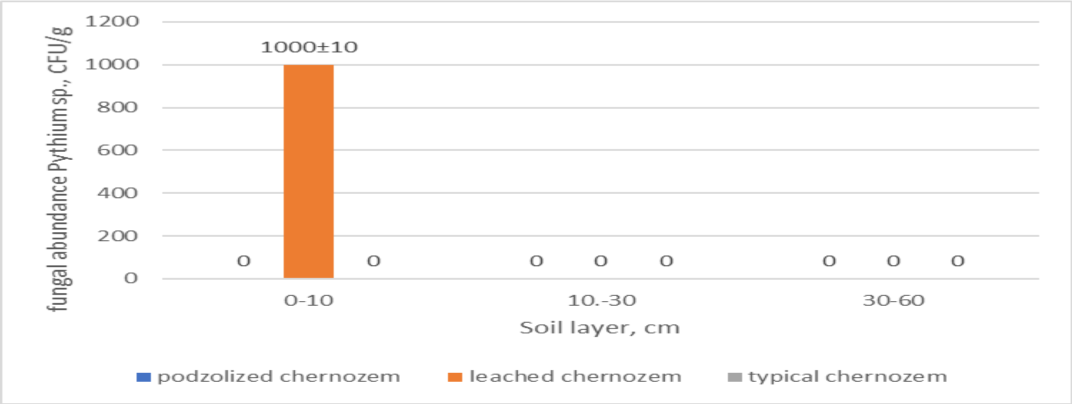

Figure 5. Abundance of *Pythium* spp. fungi in the rhizosphere of apple trees in intensive orchards, depending on the soil subtype (CFU/g).

Table 1. Agrochemical characteristics of the studied soils in apple orchards.

Soil indicator	Soil subtype and sampling depth (cm)								
	Podzolized chernozem			Leached chernozem			Typical chernozem		
	0-10	10-30	30-60	0-10	10-30	30-60	0-10	10-30	30-60
pH_{KCl}	5.2±0.1	5.1±0.1	5.0±0.1	5.5±0.1	5.7±0.1	5.8±0.2	5.9±0.2	6.6±0.2	6.9±0.2
Hydrolytic acidity (mg equivalent/ 100 g of soil)	9.9±0.3	10.2±0.3	11.0±0.3	9.7±0.3	7.3±0.3	4.0±0.1	3.5±0.1	2.0±0.1	0.5±0.1
Humus content (%)	4.7±0.1	4.5±0.1	3.7±0.1	5.7±0.2	5.6±0.1	4.3±0.1	6.2±0.2	5.4±0.1	4.1±0.1
The sum of the exchange grounds (mg equivalent/ 100 g of soil)	36.1±2.3	37.2±2.5	38.7±2.0	38.2±2.0	39.6±2.1	42.5±2.3	44.0±2.4	65.9±3.2	66.8±4.0
Cation exchange capacity (mg equivalent/100 g of soil)	46.0±2.5	47.4±2.8	49.7±2.8	47.9±2.7	46.9±2.5	46.5±2.5	47.5±2.8	67.9±3.3	67.3±3.4
Degree of saturation with bases (%)	78.4±4.6	78.5±4.8	77.8±4.4	79.7±4.5	87.4±5.0	91.4±5.1	92.6±5.2	97.0±5.3	99.2±5.5
Nitrogen is easily hydrolyzable (mg/100 g of soil)	9.5±0.4	9.0±0.4	8.2±0.3	10.2±0.4	9.5±0.4	8.1±0.3	13.0±0.5	12.7±0.5	11.0±0.5
Mobile phosphorus (mg/100 g of soil)	6.7±0.2	5.1±0.1	3.7±0.1	11.2±0.3	10.0±0.3	7.6±0.2	18.7±1.0	9.2±0.3	5.5±0.1
Exchangeable potassium (mg/100 g of soil)	16.0±0.6	14.4±0.6	12.0±0.5	22.0±1.1	13.6±0.5	10.1±0.5	26.8±1.2	17.8±1.0	10.9±0.5

The accuracy of the experiment ranged (P) from 4% to 7%.

RESULTS AND DISCUSSION

Soil subtype and depth influence on fungal genera

The main agrochemical characteristics of soil subtypes, depending on the depth of sampling, are available in Table 1. The accuracy of the experiment ranged (P) from 4% to 7%. The results of microbiological analysis revealed a significant positive correlation between the distribution and abundance of certain fungal genera and the soil subtype, especially due to changes in soil acidity (pH) and the presence of carbonate horizons.

Acidophilic and neutralophilic genera

Fungi associated with acidic conditions pH_{KCl}

The *Penicillium* sp. population significantly increased with depth, reaching its maximum value only in the podzolized chernozem (r = 0.95), which was the most acidic soil subtype and represents a historically native environment for apple growth (Zakharov, 2021; Figure 1). Conversely, the number of *Penicillium* sp. reached a sharp decrease with depth in the leached (r = -0.60) and typical chernozem (r = -0.93), and the latter was the most alkaline section due to the shallow depth of the carbonate horizon. The significant positive correlation between *Penicillium* abundance and soil acidity (r = 0.99) revealed the said genus was highly acidophilic.

The fungi preference for lower pH environments was consistent with past findings of Bridge and Spooner (2001), who also reported that numerous filamentous fungi, including *Penicillium*, dominate in the acidic forest and subforest soils. The highest biomass was prevalent in podzolized chernozem, which may also be linked to the accumulation of specific organic acids in this less-cultivated soil, eventually promoting the fungal growth (Al-Zubaidi and Al-Taie, 2022).

Table 2. Number of fungi in soils, not graphically represented (thousands CFU/g).

Genera of fungi	Soil subtype and sampling depth (cm)								
	Podzolized chernozem			Leached chernozem			Typical chernozem		
	0-10	10-30	30-60	0-10	10-30	30-60	0-10	10-30	30-60
Nigrospora sp.	9.0	10.0	44.0	0	0	0	0	3.1	10.94
Fusarium sp.	1.0	2.5	15.0	2.6	6.97	0.85	2.5	1.75	4.03
Botrytis sp.	0	2.0	3.9	0.37	0	0	0.7	0	0
Rhizopus sp.	1.0	3.0	4.1	1.07	0.81	0	3.4	4.76	0
Trichoderma sp.	4.8	30.0	90.0	0	0.5	0.33	3.52	4.49	2.7
Alternaria sp.	0	0	0	1.0	0	0	1.0	1.0	1.0
Cladosporium sp.	0	0	0	3.5	0	0	0	0	0

The accuracy of the experiment ranged (P) from 3% to 5 %. The least significant difference = 20 CFU/g.

The largest population of *Nigrospora* sp. (Table 2) and *Verticillium* sp. were evident in the most acidic soil subtype, podzolized chernozem (Figure 4). Their abundance indicated sharp and steady increases with the soil depth (r = 0.92 and r = 0.99, respectively). In more alkaline soil subtypes, these fungi were either absent or present in extremely low numbers (e.g., no *Nigrospora* sp. cells appeared in the 0–60 cm layer of leached chernozem). The almost exclusive dominance of fungi *Nigrospora* and *Verticillium* in the podzolized chernozem, paired with a high negative correlation with pH_{KCl} (both r ~ -0.95), suggested these fungi were highly sensitive to alkalinity.

The restriction of fungi *Verticillium* and *Nigrospora* to acidic environments supported the idea that soil pH acts as a dominant selective pressure. Deakin *et al*. (2018) also highlighted the same pattern of microbial communities in their study of apple orchards. In alkaline soils, their sharp decline differentiates them from cosmopolitan genera, confirming their preference for the specific and less-buffered edaphic niche of podzolized soils (Kichu *et al.,* 2019).

Genera tolerant to alkaline conditions

Fungi species *Mucor* appeared to be more tolerant to higher pH environments than the *Penicillium* sp. A stable increase in *Mucor* sp. with soil depth was noteworthy in both podzolized (r = 0.99) and leached (r = 0.97) chernozems (Figure 2). However, in the most carbonate-rich soil subtype, the typical chernozem was evident with a noticeable decrease (r = -0.72) in this fungi with the soil depth. However, *Mucor* sp. reached a wide distribution; the significant correlation with decreasing pH (r = -0.91 with pH_{KCl}) suggested that it still prefers the less alkaline soil conditions.

The widespread moderate sensitivity of *Mucor* sp. to high pH gained general recognition in studies of arable soils (Rodrigues *et al.,* 2014). The ability of fungi *Mucor* sp. to thrive in the intermediate leached chernozem, where pH was near neutral, suggested a broad nutritional profile. However, the sharp decline in the carbonate-rich typical chernozem confirmed that extreme alkalinity proved to be a limiting factor for its proliferation (Temjen *et al.,* 2022).

The abundance of fungi *Aspergillus* sp. decreased with soil depth only in the most alkaline soils, such as typical chernozem (r = -0.95) (Figure 3). In the podzolized chernozem, the *Aspergillus* sp. population increased with soil depth (r = 0.80). However, its lowest concentration resulted in the humus horizon of the typical chernozem. *Aspergillus* was generally popular for its high adaptability across diverse soil environments. Its negative correlation with depth only in the most carbonate soil subtype (r = -0.95) suggested it can tolerate a wide pH range, with the highest alkalinity found in the typical chernozem deep layers still limiting its proliferation.

The ubiquitous nature of *Aspergillus* often allows it to colonize both acidic and neutral soils (Shelton *et al.,* 2022). However, its limited presence in the most alkaline horizon, as observed in the typical chernozem, suggested the high buffering capacity provided by carbonates eventually restricts even this adaptable genus, mostly requiring access to decaying organic matter near the soil surface (Caputo *et al.,* 2015).

Both *Cladosporium* sp. (Table 2) and *Pythium* sp. emerged exclusively in low numbers in the leached chernozem, where their abundance further decreased with soil depth (r = -0.80) (Figure 5). These fungi were undetectable in the other soil subtypes in the 0–60 cm soil layer. The restriction of these genera primarily to the intermediate leached chernozem suggested a requirement for a specific, narrow pH range and moisture regime characteristic of this soil subtype.

The specific habitat requirement observed for *Cladosporium* and *Pythium* revealed their presence highly coupled with the unique pH and moisture regime of the leached chernozem, which significantly differed from the considerable acidic podzolized and highly buffered typical soil subtypes (Franke-Whittle *et al.,* 2015). These results occurred consistent with past studies revealing that specific fungal groups showed strict confinements from narrow ecological niches (Crous *et al.,* 2021).

Correlation analysis with edaphic factors

The degree of correlation between the number of fungi and agrochemical parameters of soils appears in Table 3. A consistent and considerable inverse relationship was evident between soil pH_{KCl} and the abundance of nearly all soil fungi. As the pH decreased (and acidity increased), the cells' content of all 12 investigated genera significantly increased. The most acid-sensitive genera showed the highest negative correlation with pH_{KCl}, such as *Penicillium*, *Nigrospora*, *Fusarium* (Table 2), and *Verticillium*, and exhibited correlation coefficients ranging from r = -0.95 to -0.99. The remaining genera, such as *Mucor*, *Aspergillus*, *Botrytis*, *Rhizopus*, *Trichoderma*, *Alternaria* (Table 2), *Cladosporium*, and *Pythium,* also displayed significant negative correlation with pH_{KCl}, ranging from r = -0.50 to -0.97. A corresponding analysis with hydrolytic acidity disclosed a uniformly considerable positive correlation for all 12 genera (*Penicillium*: r = 0.5 to 0.99; *Mucor*: r = 0.86 to 0.97).

This clear and robust evidence revealed the podzolized chernozem, characterized by its highest acidity, offers the most favorable chemical environment for the development of most fungal genera in the apple tree rhizosphere. The overwhelming negative correlation between fungal abundance and soil pH was a well-established pattern in temperate agricultural systems, exhibiting that soil acidity was the most critical chemical constraint (Xu *et*

Table 3. Relationship between some agrochemical parameters of the soil and the number of soil fungi.

Genera of fungi	The degree of correlation of the number of fungi with the hydrolytic acidity of the soil (r)	The degree of correlation of the number of fungi with soil pHKCl (r)	The degree of correlation between the number of fungi and the humus content in the soil (r)
Penicillium spp.	0.5-0.99	-0.95…-0.99	0.6-0.8
Nigrospora spp.	0.6-0.91	-0.95…-0.99	0.65-0.74
Verticillium spp.	0.6-0.91	-0.95… -0.99	0.7-0.85
Mucor spp.	0.86-0.97	-0.50 …-0.97	0.6-0.8
Aspergillus spp.	0.6-0.91	-0.50…-0.97	0.7-0.85
Fusarium spp.	0.6-0.91	-0.95…-0.99	0.65-0.74
Botrytis spp.	0.6-0.91	-0.50…-0.97	0.7-0.85
Rhizopus spp.	0.6-0.91	-0.50…-0.97	0.6-0.8
Trichoderma spp.	0.6-0.91	-0.50…-0.97	0.7-0.85
Alternaria spp.	0.6-0.91	-0.50…-0.97	0.7-0.85
Cladosporium spp.	0.6-0.91	-0.50…-0.97	0.6-0.8
Pythium spp.	0.6-0.91	-0.50…-0.97	0.7-0.85

al., 2022). This robust relationship suggested the higher soil acidity in the podzolized chernozem, coupled with high organic matter, provides optimal conditions for fungal dominance, confirming that soil chemistry is the predominant factor governing the fungal community composition in these apple orchards (Wang *et al.,* 2024).

CONCLUSIONS

The findings demonstrated the distribution and abundance of soil fungal genera in the rhizosphere of 12-year-old intensive apple orchards were considerably dependent on the chernozem soil subtype and associated soil chemistry. The podzolized chernozem, as characterized by the highest diversity and total fungal population, exhibited a positive relationship between the soil depth and key genera population, including *Penicillium*, *Fusarium*, *Botrytis*, and *Verticillium*. Conversely, the more alkaline soils, such as typical chernozem and leached chernozem, showed a decrease in fungal abundance with increasing depth for most genera. The study also confirmed that soil acidity (low pH) and high humus content served as the primary edaphic factors supporting the greater population and diversity of fungi across the three soil subtypes. Farming methods for intensive apple cultivation should consider the specific subtype of chernozem soils, since the humus content and the level of soil acidity significantly affect the composition of the fungal community of the rhizosphere. This is vital for the overall health of the garden. The data obtained can benefit in predicting the danger of soil contamination with various diseases, for example, verticillium wilt, which is very dangerous for black and red currants.

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