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Research Article

Effects of Invasive Plants(*Xanthium spinosum, Xanthium strumarium* and *Phragmites australis)* on Rhizobacteria Density

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Abstract: This study investigated whether certain invasive plant species affect the densities of soil bacteria essential for plant growth in agricultural regions. In this study, the densities of nitrogen-fixing (NF), phosphate-solubilizing (PS), and siderophore-producing bacteria (SPB) were assessed in the rhizospheres of the invasive plants *Xanthium spinosum, Xanthium strumarium*, and *Phragmites australis* in wheat cultivation areas of Çorum, Turkey. The microbial densities in the rhizospheres of these invasive plants were then compared to those found in the rhizospheres of wheat. The research findings indicated that the rhizosphere microbial density of invasive plants varied according to plant type and localit, with these variations between the wheat rhizosphere soil and those of other plant types at the same location showing statistical significance($p \leq 0.05$). However, when comparing the biochemical properties of invasive plants and wheat rhizosphere soil, no statistically significant difference was observed. The results suggest that invasive plants such as *X. spinosum*, *X. strumarium*, and *P. australis* reduce the microbial density of NFB, PSB, and SPB in environments with similar soil properties. Nonetheless, further research is necessary to deepen the analysis of the relationship between plant species and soil structures to better understand the mechanisms behind the decline in bacterial density.

Keywords: invasive plants; rhizosphere bacteria; xanthium spinosum; xanthium strumarium; phragmites australis

Araştırma Makalesi

Bazı İstilacı Bitkilerin (*Xanthium spinosum, Xanthium strumarium* ve *Phragmites australis*) Kök Bakterileri Yoğunlukları Üzerine Etkileri

Özet: Bu çalışmanın amacı, bazı istilacı bitki türlerinin tarımsal bölgelerde bitki büyümesi için gerekli olan belirli toprak bakterilerinin yoğunluklarını etkileyip etkilemediğini araştırmaktır. Bu araştırmada, Türkiye'de Çorum ilinde, buğday ekim alanlarında görülen istilacı bitkiler *Xanthium spinosum, Xanthium strumarium* ve *Phragmites australis* 'in rizosfer toprağında azot bağlayıcı (NF), fosfor çözücü (PS) ve siderofor üreten bakterilerin (SPB) yoğunlukları belirlenmiştir. Bu istilacı bitkilerin rizosferindeki mikrobiyal yoğunluklar daha sonra buğday bitkisinin rizosfer toprağındaki aynı özellikteki bakteri yoğunluğu ile karşılaştırılmıştır. Araştırma bulguları, istilacı bitki-

lerin rizosfer mikrobiyal yoğunluğunun bitki türüne ve lokaliteye göre değiştiğini ve bu mikrobiyal yoğunluğun buğday rizosfer toprağı arasında aynı lokasyon içinde istatistiksel olarak anlamlı olduğunu göstermiştir ($p \leq 0.05$). Bununla birlikte, istilacı bitkiler ile buğday rizosfer toprağının biyokimyasal özellikleri karşılaştırıldığında, istatistiksel olarak anlamlı bir fark gözlenmemiştir. Çalışma sonucunda, *X. spinosum, X. strumarium* ve *P. australis* gibi istilacı bitkilerin benzer toprak özelliklerine sahip ortamlarda bitki gelişimi için önemli olan NFB, PSB ve SPB'nin mikrobiyal yoğunluğunu azaltabileceğini göstermiştir. Bununla birlikte, bakteri yoğunluğundaki düşüşün arkasındaki mekanizmaları daha iyi anlamak için bitki türlerinin ve toprak yapılarının analizini derinleştirmek için daha fazla araştırma yapılması gerekmektedir.

Anahtar Kelimeler: istilacı bitkiler; rizosfer bakterileri; *xanthium spinosum; xanthium strumarium; phragmites australis*

1. Introduction

The introduction of non-native plant species poses a significant challenge in the context of global human-induced environmental changes. The invasion of various plant species substantially reduces the diversity and abundance of plant and animal populations [1].Additionally, invasive plants can disturb the activity, biomass, and composition of soil bacteria and other microorganisms, thereby affecting critical ecological processes such as organic matter decomposition and nutrient cycling [2]. Therefore, understanding the interactions between invasive plants and soil is crucial for identifying the mechanisms of infestation [3]. Invasive plant species release chemicals, such as phenolics, hydroxamic acids, and short-chain fatty acids, which exhibit inhibitory activity against non-adapted plant species [4]. These chemicals can significantly affect important soil microorganisms. Plant growth-promoting bacteria (PGPB) directly affect plant growth and soil fertility protection through various mechanisms, such as nitrogen fixation, phosphorus solubilization, iron chelation, and phytohormone production. Nitrogen-fixing (NF) bacteria form symbiotic relationships with different plants and aid in atmospheric nitrogen fixation. The ability of bacteria to solubilize phosphate is a significant feature of plant phosphate nutrition. Siderophores, low-molecular-weight iron-chelating secondary metabolites produced by diverse groups of microorganisms, contribute to iron scavenging under iron-limited conditions. Siderophores produced by various bacteria support plant growth by supplying iron to plants [5]. A study was observed that *Alliaria petiolata*, the most invasive plant in North America, suppressed the mycorrhizal fungi of native plants[6]. This negative impact on the fungi was attributed to the presence of various flavonoids and other compounds manufactured by Alliaria petiolata. Based on field research, it has been found that the invasive plant Reynoutria japonica negatively impacts soil microbial groups and processes such as C and N mineralization [7]. Conversely, some studies have shown that Solidago canadensis, another invasive plant, influences the structure of soil nitrogen-fixing bacteria and enhances soil nitrogen availability, thus aiding in its spread [8]. In Çorum, Turkey, *X. spinosum, X. strumarium*, and *P. australis* are the most aggressive and successful invaders. In a study was identified phytotoxic compounds from *X. spinosum*, including xanthatin, 1α,5α-epoxyxanthatin, 4-epiisoxanthanol, 4-epixanthanol, loliolide, and dehydrovomifoliol[9]. The other study was identified cytotoxic compounds from *X. strumarium*, including squalene, xanthatin, stigmasterol, and β-sitosterol-O-glucoside[9]. Moderate cytotoxic alkaloids, including phranisines, N-p-coumaroyl serotonin, and N-p-coumaroyl tryptamine, have been extracted from the roots of *Phragmites australis* [11]. However, limited information is available on how invasive plant species alter the microbiological structure of soil. Therefore, it is particularly important to investigate whether invasive plant species impact the populations of soil bacteria.

The primary objective of this study was to evaluate the density of bacterial populations present in the rhizospheres of invasive plant species. Additionally, this study compared the microbial density associ-

ated with invasive plant species to that found in the wheat rhizosphere. By analyzing these two distinct environments, we aimed to gain insights into how invasive plants influence microbial communities in soil, potentially affecting ecological balance and agricultural practices. This study provides important information on the interactions between invasive species and soil health, with implications for environmental management and crop production. We hypothesized that the bacterial populations in wheat rhizosphere soil differ among the rhizospheres of *X. spinosum, X. strumarium,* and *P. Australia*. The soil bacterial population will generally be lower in the rhizospheres of *X. spinosum, X. strumarium*, and *P. australis* than in wheat rhizospheres.

2. Materials and Methods

The invasive species *X. spinosum* (Asteraceae), *X. strumarium* (Asteraceae), and *P. australis* (Poaceae) were collected from five different locations in wheat fields located in Çorum province (Table 1). *Xanthium spinosum* is a vertically growing, rigid, highly branched annual plant, reaching lengths of 30-100 cm and occasionally reaching 150 cm. The stems are ribbed, yellowish or gray-brown, and thinly hairy. *Xanthium strumarium* is an annual plant that typically grows 30–100 cm in height. It features erect, branched stems that are often speckled with purple and adorned with short white hairs scattered along the surface [12]. *Phragmites australis* typically reaches heights of 2–4 m. Its leaves are 20–50 cm long and 2–7 cm wide. During late summer, the tassels are striking dark purple, approximately 20–30 cm long [13](Figure 1).

Table 1. Invasive plant location and location coordinates using rhizosphere soil in Corum

Locality Number	Location Coordinates	Variety of Species	Location
Locality 1	40º26’54”N-34º53’17”E	*Phragmites australis*	Wheat in the field
Locality 2	40º32’31”N-34º56’10”E	*Phragmites australis*	wheat field border
Locality 3	40º32’38”N-34º59’27”E	*Xanthium spinosum*	Wheat in the field
Locality 4	40º32’33”N-34º59’20”E	*Xanthium spinosum*+ *Xanthium strumarium*	Wheat field border
Locality 5	40º32’52”N-34º 59’11”E	*Xanthium strumarium*	Wheat in the field

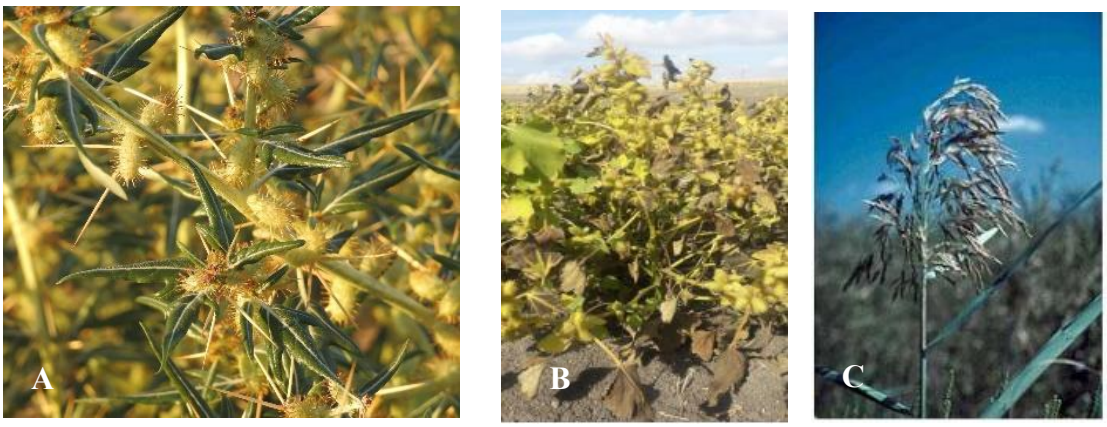

Figure 1. Above ground parts of the invasive plant spcies used in this study: A) X. spinosum; B) X. strumarium; C) P. australis.

2.1. Soil Sampling

Soil samples were collected from the rhizospheres of both the invasive plant species and wheat plants from each of the five wheat planting locations. Soil samples were randomly collected from the rhizosphere region of each plant at a depth of 20 cm [14]. Three replications were performed at each location. The rhizosphere soils were sifted using a 2-mm mesh and transported to the laboratory in May 2022. The rhizosphere soil was stored at 4°C for one day to isolate bacteria and to determine its biochemical properties.

2.2. Isolation of Bacteria

2.2.1.Soil Preparation by Dilution

The bacterial strains from rhizosphere soil samples were isolated using dilution plate techniques[15].To isolate bacteria from soil, 10 g of each soil sample was suspended in 90 mL of 0.85% m/V NaCl, agitated at 120 rpm for 30 min, diluted 1:10, and stored at 4°C. Subsequently, a dilution series of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} were prepared.

2.2.2. Isolation of Phosphate-Soluble Bacteria

100 µL of each dilution was applied to NBRIP agar plates with the following composition per liter: 10 g glucose, 5 g $Ca_3(PO_4)_2$, 5 g $MgCl_26H_2O$, 0.25 g $MgSO_47H_2O$, 0.2 g KCl, 0.1 g $(NH_4)2SO_4$, and 1.5% agar, pH 7. After 10 days, bacterial colonies around the clear halo zone were counted using a colony counter. After 10 days, bacterial colonies with clear transparent zones around the periphery were counted. This indicates that the bacteria were able to solubilize phosphate, as evidenced by the formation of a clear zone around the colony. This method is commonly used to quantify the phosphate-solubilizing bacteria (PSB) population in soil or other samples.

2.2.3. Isolation of Nitrogen-Fixing Bacteria

The bacteria were isolated using Döbereiner nitrogen-free agar medium, which comprised 5.0 g malic acid, 5 g $C_{12}H_{22}O_{11}$, 0.4 g $KH_2PO_4 \cdot H_2O$, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.1 g NaCl, 0.02 g $CaCl_2 \cdot H_2O$, 0.01 g $FeCl_3$, 0.002 g $Na_2MoO_4 \cdot 2H_2O$, 0.05 g/L bromothymol blue, and 15.0 g/L agar–agar at pH 7.0. After inoculating soil with 100 µl of dilutions, the samples were incubated at 30°C for 72 h on N-free agar (NFA) in triplicate. After 72 h of incubation, we meticulously tallied the individual bacterial colonies that transitioned from yellow to a distinct bluish hue. This process allows us to accurately assess nitrogen-*fixing* bacterial counts.

2.2.4. Isolation of Siderophore-Producing Bacteria

Siderophore-producing microorganisms were counted using Chrome Azurol S (CAS) medium plates. A 100 µl dilution of the sample was inoculated onto the plates, which were then incubated at 37 °C for 72 h[16].After the incubation period, the bacterial isolates that caused a color change in the medium from blue to orange-yellow were carefully counted. All bacterial colony-forming units were determined using the following formula:

$$\textbf{Colony Forming Unit(CFU)}\ \mathbf{g^{-1}} = \textbf{number of colonies x dilution factor} \quad (2.1)$$

The log CFU/mL was calculated using:

$$\textbf{Log}\left(\frac{\textbf{CFU}}{\textbf{ml}}\right) = \textbf{log10}\,\frac{\textbf{CFU}}{\textbf{dilution factor x volume(ml)of culture plated}} \quad (2.2)$$

2.3. Statistical Analysis

The statistical analysis of the data was performed using SPSS 20.0 software. An independent samples t-test was specifically employed to ascertain the presence of statistically significant differences between the bacterial counts and in the dataset and their mean values ($p \leq 0.05$).

3. Results and Discussion

Soil plays a vital role in supporting microbial life in the environment. Many studies have shown that the interaction between plant species and microbial life is influenced by the combination of plant species and soil physicochemical properties, such as soil pH. Our findings suggest that specific invasive plant species have a significantly adverse impact on soil bacterial diversity. Comprehensive soil analyses conducted at each locality, no statistically significant differences in the biochemical values between the invasive plant and the rhizosphere soil of the wheat plant in the same locality were revealed during these analyses. This suggests that the biochemical compositions of the invasive plant and the rhizosphere soil of the wheat plant are similar, indicating a potential lack of significant impacts on soil biochemistry by the invasive plant in this specific context (Table 2). The bacteria in the rhizosphere soil samples of invasive plants exhibited lower population densities than those present in the non-invasive plant Triticum aestivum. This was determined by counting the bacteria in Petri dishes, as illustrated in Figure 2. Invasive plant species in all localities showed a significant difference in bacterial density in the rhizosphere soil compared with the bacterial density in wheat plant rhizosphere soil at the same locality. A significant difference was observed in the population of phosphate solubilizing, nitrogen-fixing, and siderophore-producing bacteria between the invasive plant rhizosphere and Triticum aestivum rhizosphere soil($p \leq 0.05$).

In various locations, the populations of phosphate-solubilizing bacteria (PSB) in soil samples from the rhizosphere of the invasive plant, ranged from 3.00 to 5.86 Log CFU/g. In the rhizospheres of *Triticum aestivum*, the population of PSB ranged from 6.33 to 7.65 Log CFU/g. In comparisons between invasive plants and Triticum aestivum, statistical analysis revealed a significant difference in the numbers of PSB (phosphate-solubilizing bacteria) in the rhizosphere soil of the invasive plants compared to those in the wheat rhizospheres. This difference was found to be statistically significant in each locality($p \leq 0.05$). The number of nitrogen-fixing bacteria (NFBs) isolated from the rhizosphere soil of the invasive plant in each locality was statistically different from that of the wheat plant in the same locality. This suggests that the presence of invasive plants may indeed have a notable impact on the nitrogen-fixing bacteria (NFB) microbial composition of the soil. In the rhizospheres of the control plant Triticum aestivum at locality 1, the highest number of nitrogen-fixing bacteria(NFB) (7.33 Log CFU/gr) was identified. Conversely, the rhizosphere soil of the invasive plant species *Xanthium spinosum* at locality 3 had the lowest number of NFBs (5.63 Log CFU/gr).

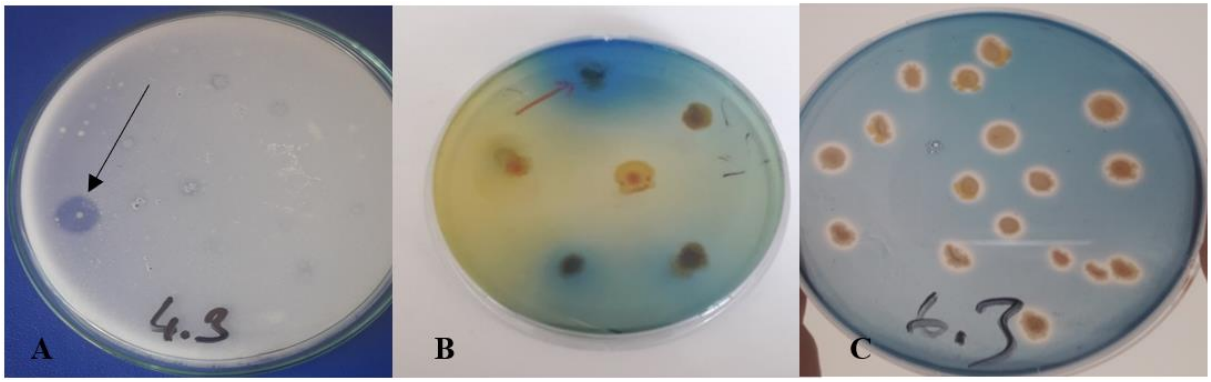

Figure 2. Bacteria isolated from rhizosphere soils of invasive plants (A:PSB,B:NFB,C:SPB)

The proportions of siderophore-producing bacteria (SPB) in the rhizospheres of invasive and the Triticum aestivum plants were determined. The SPB population densities in each locality were significantly different based on the wheat varieties($p \leq 0.05$). The siderophore-producing bacteria counts ranged from log10 3.66 to log10 5.66 cfu g−1 in the rhizosphere soils of invasive plants. In wheat rhizosphere soil, this number varied between 6.33 and 7.52 in log10 scale. These results show that the proportion of endophytic bacteria colonizing the roots of invasive plants tended to decrease (Table 2). When the biochemical properties of the soils were analyzed, we found that there were differences between them. However, these differences were not statistically significant compared with the biochemical properties of wheat rhizosphere soil from the same locality. The pH of wheat rhizosphere soil varied between 7.10 and 7.40, whereas the pH of invasive plant rhizosphere soil varied between 7.08 and 7.30. The highest potassium content (225.22 mg/kg) was found in the fifth locality, while the lowest content (110.11 mg/kg) was found in the third locality. In the invasive plant rhizosphere soil, these ratios ranged between 111.83(locality 1) and 220.7 mg/kg (locality 1). In the rhizospheres of wheat plants, the highest level of phosphorus (16.25 mg/g) was determined at locality 2, whereas in the rhizospheres of invasive plants, the highest level was 16.15 mg/g, also at locality 2. In the same locality, the nitrogen values in the wheat plant and invasive plant rhizosphere soil were comparable.

Table 2. Effects of the invasive species X. spinosum, X. strumarium, P. australis, and Triticum aestivum on PSB, NFB, and SPB, which are considered as biochemical properties of soil

Location	Species	PSB Log CFU/gr ±SE	NFB Log CFU/gr ±SE	SPB Log CFU/gr ±SE	Rhizosphere Soil Properties					
					pH	Lime	Potassium (mg/kg)	Phosphorus (mg/g)	Organic Matter (%)	Nitrogen (%)
1	*Triticum aestivum*	6.33±0,88^{a}	7.33±0.33^{a}	6.33±0.33^{a}	7.10±0.5^{a}	0.36±0.008^{a}	112.5±3.73^{a}	16.06±0.66^{a}	1.36±0.16^{a}	0.17±0.11^{a}
	Phragmites australis	3.00±0.57^{b}	4.33±0.33^{b}	3.66±0.33^{b}	7.08±0.88^{a}	0.37±0.003^{a}	111.83±4.57^{a}	15.76±0.43^{a}	1.39±0.29^{a}	0.18±0.15^{a}
2	*Triticum aestivum*	7.65±0.53^{a}	6.43±0.14^{a}	7.03±0.20^{a}	7.26±0.3^{a}	1.35±0.005^{a}	122.4±2.26^{a}	16.25±0.106^{a}	1.46±0.16^{a}	0.14±0.13^{a}
	Phragmites australis	4.33±0,00^{b}	5.03±0.42^{b}	4.46±0.45^{b}	7.21±0.12^{a}	1.33±0.002^{a}	121.8±6.66^{a}	16.15±0.20^{a}	1.49±0.29^{a}	0.16±0.08^{a}
3	*Triticum aestivum*	6.84±0.75^{a}	6.98±0.44^{a}	7.52±0.34^{a}	7.27±0.12^{a}	0.16±0.002^{a}	110.11±6.33^{a}	6.54±0.916^{a}	1.41±0.03^{a}	0.08±0.01^{a}
	Xanthium spinosum	5.16±0.66^{b}	5.63±0.17^{b}	5.16±0.34^{b}	7.30±0.45^{a}	0.20±0.001^{a}	114.8±5.32^{a}	6.15±0.252^{a}	1.46±0.09^{a}	0.06±0.05^{a}
4	*Triticum aestivum*	7.04±0.55^{a}	7.18±0.14^{a}	6.83±0.45^{a}	7.26±0.42^{a}	1.18±0.025^{a}	118.37±22.89^{a}	6.206±0.326^{a}	1.49±0.32^{a}	0.14±0.22^{a}
	Xanthium strumarium	5.86±0.74^{b}	5.32±0.28^{b}	5.14±0.32^{b}	7.20±0.11^{a}	1.21±0.013^{a}	120.8±5.32^{a}	6.26±0.452^{a}	1.40±0.25^{a}	0.16±0.25^{a}
5	*Triticum aestivum*	7.23±0.35^{a}	6.45±0.34^{a}	7.23±0.34^{a}	7.46±0.19^{a}	15.59±0.027^{a}	225.22±9.14^{a}	10.025±1.196^{a}	0.43±0.03^{a}	0.04±0.01^{a}
	Xanthium strumarium	5.20±0.36^{b}	5.17±0.30^{b}	5.66±0.85^{b}	7.42±0.24^{a}	15.45±0.042^{a}	220.7±6.41^{a}	9.254±0.965^{a}	0.41±0.15^{a}	0.06±0.21^{a}

*Values represent the mean of 3 replications. Means followed by different letters in each locality and column are significantly different at $p \leq 0.05$.(PSB, phosphate solubilizing bacteria; NFB, nitrogen-fixing bacteria; SPB,siderophore-producing bacteria)

Soil microorganisms, including bacteria, fungi, and archaea, play crucial roles in promoting plant growth in agricultural fields. These microorganisms contribute to various key processes, such as nutrient cycling, where they help in the process of decompose organic matter, thereby making essential nutrients like nitrogen, phosphorus, and potassium more available to plants. They also enhance soil structure and health by forming symbiotic relationships with plant roots, such as mycorrhizal associations that improve water and nutrient uptake. In addition, certain soil microbes can suppress plant diseases by outcompeting harmful pathogens or producing natural antibiotics. Overall, the presence and diversity of soil microorganisms are vital for optimizing plant health and increasing agricultural productivity. This study investigated the influences on soil microbial activity of three invasive plant species: *X. spinosum, X. strumarium,* and *P.australis.* The results were compared with those of the cultivated plant Triticum *aestivum*. The study conducted a comprehensive analysis demonstrating that microbial densities were significantly negatively impacted by the presence of invasive species, gibiting distinct differences when compared with the effects observed in wheat cultivation. The research highlighted how these invasive species disrupt the balance of microbial communities in the soil, leading to varied ecological outcomes that could have implications for soil health and agricultural productivity. In this study, we observed that the presence of this specific invasive plant species adversely affects the densities of soil bacteria within the same ecosystem. This negative impact may be linked to the competitive and antagonistic interactions between various invasive plant species. These interactions can disrupt the natural balance of soil microbiota, leading to reduced bacterial populations essential for maintaining soil health and fertility. Consequently, our findings suggest that the complex dynamics among different invasive plants can significantly influence the overall microbial community structure in soil, thereby affecting ecological functions. The relationship between invasive species diversity and soil microbial diversity can be attributed to the ways in which invasive species alter the physicochemical properties of soil. For instance, when invasive plants or organisms establish themselves in a new environment, they modify factors such as soil pH, nutrient levels, moisture content, and organic matter composition. These changes can, in turn, affect the microbial communities in the soil, potentially leading to shifts in microbial diversity and function. However, it is important to note that not all studies agree on this topic. Some studies have demonstrated that the introduction of invasive species does not significantly affect the physicochemical properties of soil. In these cases, invasive species may coexist with native flora and fauna without substantially altering the soil environment. This discrepancy highlighted the complexity of ecological interactions and suggested that the impact of invasive species may vary depending on specific conditions and contexts. In our study, we obtained similar results. Therefore, invasive species diversity may not lead to changes in soil physicochemical properties through alterations in soil microbial diversity. The influence of invasive plant species on soil microbial diversity observed in this study could be linked to antagonistic interactions among various invasive plants, which impact the soil microbial community. This antagonism may manifest in several ways, such as competition for resources, the release of allelopathic compounds, or altering the environmental conditions within the soil, thereby creating a less hospitable habitat for native microbial populations. Consequently, these complex interactions may lead to microbial diversity shifts, potentially disrupting the ecological balance and function of soil ecosystems. Recent research has shown that phenolic compounds in invasive plants alter soil microorganism density and biochemical structure. Root secretion of secondary metabolites may play an important role as allelopathic agents and mediators in plant–soil microbial interactions, thereby potentially changing the rate of soil processes and microbial densities in soil [17]. The phytoxic compounds of X. spinosum were identified as xanthatin, 4-epiisoxanthanol[18], 1α,5α-epoxyxanthatin, loliolide, and dehydrovomifoliol[19]. A similar type of toxic substance, xanthatin, has been identified in solvent extracts of X. strumarium leaves that is antagonistic to several bacteria and yeasts [20]. A study reported that gallic acid (3,4,5 trihydroxybenzoic acid), a chemical secreted by the roots of *P. australis*, causes cell death[21].In line with our hypothesis, we found that invasive plants negatively affect soil bacteria. In our study, these compounds may have reduced the density of bacteria in the soil, and interactions may depend on the type of invader species. Invasive plants can alter soil bacterial density. Our study is consistent with previous findings regarding the effects of invasive plants on soil microbial biomass including reduced microbial biomass.

Acknowledgement

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Conflict of Interest

The authors report no conflict of interest relevant to this article.

Research and Publication Ethics Statement

The authors declare that this study complies with research and publication ethics.

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