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Recent advances in restoration, preservation and eco-morphophysiology of plants under integrated management approaches and current climate change

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Recent advances in restoration, preservation and eco-morphophysiology of plants under integrated management approaches and current climate change

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Editorial: Recent advances in restoration, preservation, and eco-morphophysiology of plants under integrated management approaches and current climate change

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Editorial on the Research Topic

Recent advances in restoration, preservation and eco-morphophysiology of plants under integrated management approaches and current climate change

Integrated forest management has become a critical strategic action considering forests' multiple roles in nature conservation, renewable energy strategies, climate change mitigation, and adaptation policies (Sotirov and Arts, 2018). It generally involves considering the totality of interactions of various sub-systems (social, economic, and ecological) within the biosphere and integrating goals set for such management (Aggestam et al., 2020).

Ecological drivers for tree restoration and preservation include biotic and abiotic factors, such as climate, type of substrate, and site aspect (Santoyo et al., 2017) and interactions with other species of a given plant community, animals or soil microorganisms (Asbeck et al., 2021). Morphological and physiological approaches seek to understand better how species cope with variations in given conditions and resources and how organisms' responses affect their distribution and abundance patterns, community structure, and ecosystem processes (Walthert and Meier, 2017; Marchi et al., 2018).

The ecological integrity of natural ecosystems significantly impacts social well-being and sustainable development. Therefore researchers pay more and more attention to restoration and preservation approaches (Bolte et al., 2009; Nagel et al., 2017). Forest and agriculture resources could considerably support sustainable development while ensuring that natural ecosystems are restored, resilient, and protected. Therefore, understanding how various integrated management approaches affect forest ecological and morphophysiological responses is fundamental to any sound prediction regarding restoration and preservation across all the terrestrial biomes worldwide. This Research Topic gathers different contributions emphasizing advances in conservation and restoration and methods to deal more efficiently with simulated and factual data, highlighting the different approaches concerning sustainability and the integrated management of forest resources. Understanding the basis of the interaction of restoration-integrated management approaches is imperative for sustainable agriculture and forest productivity.

The articles published in this Research Topic include: 1. “Root system-rhizosphere soil-bulk soil interactions in different Chinese fir clones based on fungi community diversity change” by Cao et al.; 2. “Impact of land use change on habitat quality and regional biodiversity capacity: Temporal and spatial evolution and prediction analysis” by Li et al.; 3. “Deficit irrigation scheduling with mulching and yield prediction of guava (*Psidium guajava* L.) in a subtropical humid region” by Jat et al.; 4. “A sequential game-play modelling on forest-title mortgage loans based on Chinese forester resource and assets valuation” by Xu et al.; 5. “Phosphorus extractability in relation to soil properties in different fields of fruit orchards under similar ecological conditions of Pakistan” by Bibi et al.; 6. “Combined evaluation of corporate ecological and environmental responsibility: Evidence for forest preservation from Chinese forestry companies” by Long et al.; 7. “Interactive effects of intercropping and mulching under conservation tillage as sustainable agriculture increased cotton productivity” by Adil et al.; 8. “Effects of nitrogen addition and seasonal change on arbuscular mycorrhizal fungi community diversity in a poplar plantation” by Peng et al.; 9. “Longitudinal section cell morphology of Chinese fir roots and the relationship between root structure and function” by Li et al.; and 10. “Role of different organic and inorganic amendments in the biofortification of iodine in *Coriandrum sativum* crop” by Tianyi et al. These articles suggest various local, national, or regional transformative integrated management approaches for sustainable development.

Cao et al. found that the arbuscular mycorrhizal fungi (AMF) richness and abundance of root and rhizosphere soil within the same Chinese fir clone plantations were significantly higher than that of bulk soil. It indicates that root exudates might activate AMF in the root system and rhizosphere soil. Based on the impact of AMF diversity and the difference in symbiosis with different clones, AMF diversity can be artificially increased. The screening of different Chinese fir clones depicts that Chinese fir clone P17 has high richness and abundance, which may be a nutrient-efficient clone of Chinese fir. Jat et al. inferred that applying deficit irrigation at 75% ETc (ETc is the crop evapotranspiration mm/day) using silver-black mulch imposed the required levels of water stress on *Psidium guajava*. This improved the yield, fruit quality, and irrigation water productivity. It could be a superior option for *Psidium guajava* cultivation in Uttarakhand’s subtropical, humid Tarai conditions and in regions with similar agro-climatic conditions.

Xu et al. stated that forest management had become a critical strategic action because of forests’ diverse role in nature conservation and bio-economic benefits. Forest-title mortgage loan plays a crucial role in easing the shortage of funds that a forester might encounter, thus protecting forest resources. Their paper proposes particular suggestions about how to raise the loan limit for the forester. It includes (1) proposals pertinent to the governmental policy support, (2) introduction of innovative credit, and (3) elaboration on how foresters could integrate their forest-resource assets by using cooperatives and launching scaled productions. Bibi et al. investigated phosphorous (P) extractability by seven extraction methods regarding soil properties in three fruit orchards. They found hydrochloric acid and diethylenetriamine pentaacetate extractants gave more extractable P than other methods. This study also indicated that soil organic matter inputs and turnover associated with orchard trees exhibited a substantial quantity of extractable P in soils. It is required to predict available P in relation to its bioavailability using these methods in contrasting soils.

Adil et al. indicated that straw mulching under conservation tillage produced better results for *Gossypium herbaceum*. However, soil analysis has shown that no-tillage and leguminous crop intercropping improved soil health indicators. Moreover, tillage, leguminous crop, and mulching interaction better responded to seed yield and harvest index. They recommended no-tillage and straw mulching to achieve higher *Gossypium herbaceum* productivity. Peng et al., using traditional morphological identification, analyzed the seasonal changes of the root Arbuscular mycorrhizal fungal colonization in poplar after six-year N addition. Using high-throughput sequencing, they detected the composition and diversity of AM fungal community in the rhizosphere soil. N addition largely influenced the root colonization rate. In contrast, seasonal change had a prominent effect on the diversity indexes of the AMF community.

Li et al. mentioned that the microwave paraffin section method combined with laser scanning confocal microscopy (LSCM) could observe the cell morphology of the longitudinal section of Chinese fir roots more clearly and precisely in a short time. This method can also provide technical reference for the observation and study of the cell morphology of other tree roots’ longitudinal sections. However, the microwave treatment time needs to be adjusted accordingly. Tianyi et al. reported that the potassium iodide (KI)-fertigated soils accumulate higher iodine than the exogenous organic iodine (OI)-fertigated soils. Soil amended with wood ash increased the iodine concentration in plant tissues. Optimum iodine addition can speed up growth development; however, excessive accumulation might be detrimental to them. Therefore, a suitable soil amendment can enhance iodine availability in soil.

Li et al. studied how land use changes could affect habitat quality in Suzhou City, Jiangsu Province, China. The temporal and spatial distribution showed a habitat degradation trend from downtown to suburban areas of Suzhou. This degradation is most common in mountainous and forest areas with highly fragmented landscapes. The habitat quality of the city has changed over time and space due to socioeconomic factors, land use changes, spatial patterns, and the natural environment, with land use having the most significant impact. Long et al. carried out a study to define the content and the measurement of corporate ecological and environmental responsibility (CEER) and the role of forestry companies in forest preservation. They reported that the particularity of ecological and environmental responsibility of forestry enterprises originates from the duality of its impact on the environment. The negative externalities are caused by operating, and forest resources bring positive externalities. Therefore, forestry enterprises should bear the dual responsibility of pollution prevention and ecological construction.

The interaction of restoration and integrated management approaches is a key process that allows a forest and other natural resources to restock after a disturbance and sustain itself. It is an inclusive process that depends on collaboration among various stakeholders, including local communities, government officials, non-government organizations, scientists, and funding agencies. Its ecological success is measured in terms of increased biological diversity, biomass, primary productivity, soil health, and the characteristic of the target ecosystem.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

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Root system-rhizosphere soil-bulk soil interactions in different Chinese fir clones based on fungi community diversity change

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The diversity of the rhizosphere arbuscular mycorrhizal fungi (AMF) community is a crucial factor affecting root-soil interaction. They can absorb carbohydrates from the host body and return the nutrient elements from the soil to the host. Using 15 Chinese fir (*Cunninghamia lanceolata* Lamb. Hook.) clones, the AMF richness, abundance and community structure in "Root system-Rhizosphere soil-Bulk soil" were obtained by Real-time quantitative PCR (qPCR) and Illumina Miseq sequencing techniques. The results showed that under the same Chinese fir clone, the total amount of AMF was in the order of rhizosphere soil > root system > bulk soil. The species diversity and uniqueness of AMF were in the order of root system > rhizosphere soil > bulk soil. There was a significant correlation between soil-available phosphorus and AMF diversity and its dominant genera and species. Regarding AMF abundance, Chinese fir clone S18 is the highest, followed by clones Y061 and P17. There was a significant difference in AMF richness among different clones, and *Glomus* was the dominant genus of AMF. The AMF species diversity of P17 and S2 in roots and rhizosphere soil was high, indicating a good symbiosis between roots and the AMF community. However, the AMF diversity of clones P11 and P41 was low, and the variation of AMF community composition in the group was small. The root-soil interaction caused the AMF community to gather in the rhizosphere but had less symbiosis present with roots. Still, the AMF diversity of the rhizosphere soil of both clones was high. There was a significant correlation between the soil-available phosphorus content and the species diversity of AMF and its dominant genera and species. In conclusion, Clone P17 has high AMF richness and abundance and forms a good symbiosis with AMF, which could be a nutrient-efficient clone of Chinese fir.

KEYWORDS

arbuscular mycorrhizal fungi, diversity and abundance, root structure, rhizosphere soil, *Cunninghamia lanceolata*

Introduction

Soil fauna is central to key ecological functions of soil ecosystems (Farooq et al., 2021a; Yan et al., 2021). They break down soil organic matter and release carbon (C), oxygen (O), nitrogen (N), and phosphorus (P) into the soil (Farooq et al., 2022). Arbuscular mycorrhizal fungi (AMF) are soil's most widely distributed microbial components. They can absorb carbohydrates from the host body and nutrient elements from the soil to supply the host. Improving the nutrient use efficiency of the host is conducive to its growth and biomass accumulation to enhance its stress resistance (Smith and Read, 2008; Smith and Smith, 2011; Tufail et al., 2021). Therefore, the symbiosis between plants and AMF is key to improving forest productivity.

Chinese fir (*Cunninghamia lanceolata* Lamb. Hook.) is the most common fast-growing timber species in subtropical China, with good material and high yield per unit area (Li Y. et al., 2021); however, the management mode of continuous planting of Chinese fir plantations for multiple generations has significantly reduced the availability of soil nutrients, and the productivity of stands was difficult to maintain for a long time (Farooq et al., 2019a). However, with the maturing of Chinese fir breeding technology, excellent breeding clones have become the main means of afforestation of Chinese fir plantation. In recent years, various studies have been carried out on using mycorrhizal symbionts to maintain the high productivity of Chinese fir plantations. Some studies have shown that AMF significantly improves the aboveground biomass of Chinese fir seedlings by promoting the absorption of nutrients (Jing et al., 2020), and AMF has made great progress in improving nutrient efficiency and its mechanism (Martin et al., 2017).

Since plant productivity in a specific ecosystem depends on the diversity of fungal symbionts, AMF diversity is an important determinant of plantation ecosystems (Heijden et al., 1998). For an individual plant, its productivity increases with the increase of AMF community diversity (Vogelsang et al., 2006). At the same time, there was a preferential association between some AMF and plant genotypes (clones or varieties) (Pivato et al., 2007), and significant differences are noticed in the impact of different kinds of AMF on plants (Heijden et al., 1998). From the perspective of the plant-soil system, the rhizosphere interaction process plays a key role in the nutrient use efficiency of the system. The diversity of the AMF community is of certain importance in maintaining the sustainable production of the system (Manoharan et al., 2017). The "host preference" of AMF was considered an important factor in determining plant community structure and ecosystem function (Heijden et al., 1998; Vandenkoornhuyse et al., 2002; Sanders, 2003). Different species of AMF have different effects on host plants' competitiveness and relative abundance (Scheublin et al., 2007). Studies have shown that even if AMF of different species appear in the same area at the same time, there are differences in root colonization ability, impact on plant performance and compatibility with existing plants (Van der Heijden et al., 1998; Helgason et al., 2002).

The special behavior of AMF involves an external interface with soil and an internal interface with the root cortex to exchange nutrients with plants. Therefore, AMF diversity may be affected differently at different interfaces. From the perspective of the "root-rhizosphere-soil" system, revealing the interaction process of the rhizosphere has become the focus of research. This also raises the question about the influence of the spatial heterogeneity of the "Root system- Rhizosphere soil-Bulk soil" system on the diversity of AMF.

Currently, most of the research mainly focuses on a single rhizosphere process; thus, in-depth research from the "Root system-Rhizosphere soil-Bulk soil" system multi-interface interaction is scarce. Moreover, the research on AMF in Chinese fir plantations mainly focuses on the influence of external factors such as nitrogen deposition, continuous planting, and afforestation density on the interface rate and community structure of AMF. Still, it does not involve the response of AMF to different Chinese fir clones, genotypes or varieties. Therefore, this paper focuses on (1) the diversity, richness and community structure of AMF in the root system, rhizosphere, and bulk soil under 15 Chinese fir clones and (2) the difference in the impact of the spatial heterogeneity of the root-rhizosphere- bulk soil on AMF diversity. We hypothesized that due to the "host preference" of AMF, there were significant

Abbreviations: BD, soil bulk density; TCPOR, total porosity; CPOR, capillary porosity; AP, available phosphorus; TP, total phosphorus; TK, total potassium; AK, rapidly available potassium; TC, total carbon.

differences in AMF community diversity among roots of different clones of Chinese fir.

Materials and methods

Overview of the study area

The research was carried out on the Shanghang Baisha state-owned forest farm in Longyan City, Fujian Province (Figure 1). The test site has an altitude of 780 m. It belongs to the subtropical monsoon climate, with an average annual precipitation of 1780 mm, an average annual temperature of 18.5°C, and a frost-free period of about 270 days. The mountain soil is mainly red. The test plots present a long strip with different Chinese fir clones planted adjacent to each other. The afforestation was carried out in the spring of 2019 using 1-year-old cuttings of Chinese fir clones collected from the Chinese Fir Engineering Technology Research Center of the State Forestry and Grassland Administration. Afforestation density was 278 plants/hm^2 for all the Chinese clones.

Soil and root sample collection

According to the research objectives, fifteen Chinese fir clones (L27, P2, P11, P17, P18, P41, S22, S2, S4, S18, S23, Y020, Y061, W2, and 302) were selected for the research. An entire plot (from uphill to downhill) was used for the study without establishing the smaller sample plot. In October 2020, all the trees in the sample plots were tested for height and diameter. Then, three healthy standard trees per clone were selected. After carefully removing the litter layer, fine roots (≤2 mm) from the standard trees were collected. The shaking soil method sampled rhizosphere soil (0–5 mm from the root's surface). To collect the bulk soil sample, the soil profiles were excavated from the upper, middle and lower slopes, and undisturbed soil samples were taken from 0 to 20 and 20 to 40 cm depth using a 100 cm^3 ring knife. Soil and root samples were put into sterile plastic bags, placed in a 4°C incubator, and returned to the laboratory. Bulk soil was divided into two parts. Chinese fir roots, rhizosphere soil and one part of bulk soil were placed in an ultra-low temperature refrigerator (−80°C) to detect the abundance and diversity of AMF. The remaining bulk soil samples were used

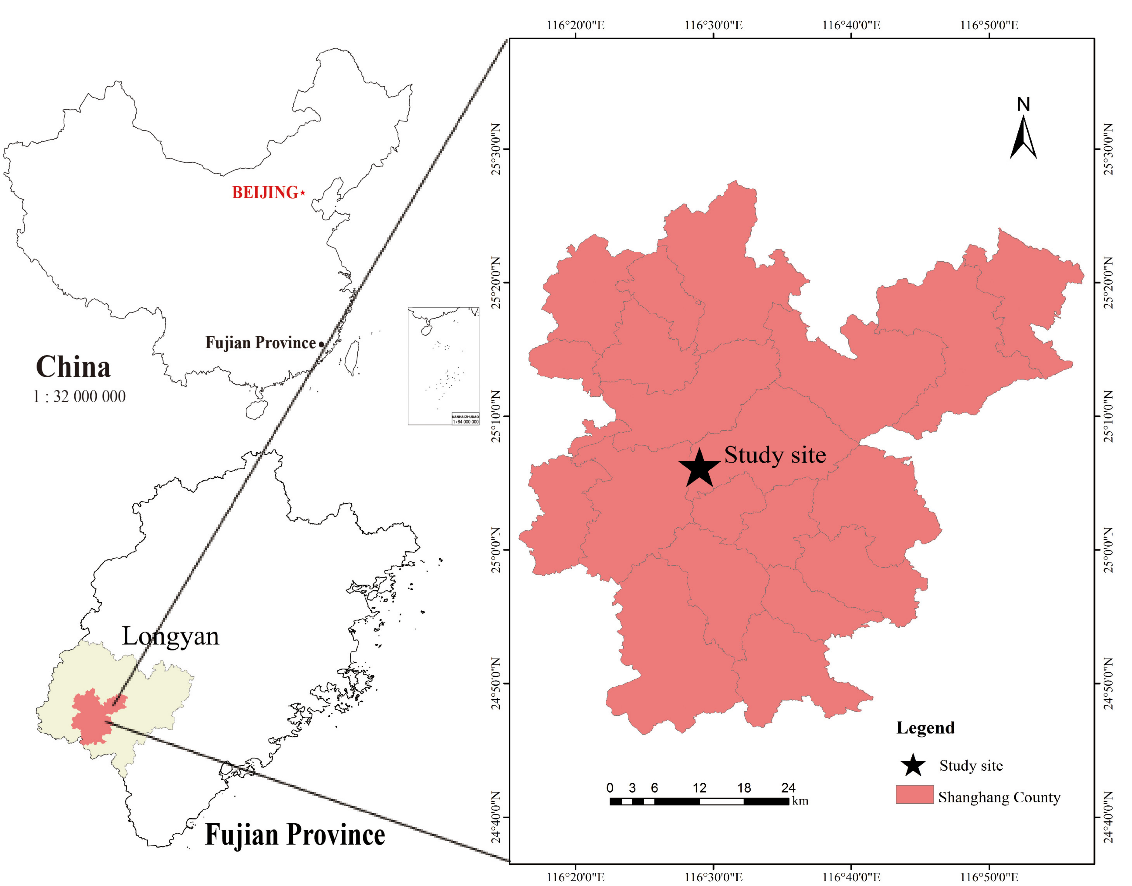

FIGURE 1
The map of the study area (Longyan City, Fujian Province, 116°37′53′′E—116°37′59′′E, 25°09′46′′N—25°09′51′′N).

to determine the soil's physical and chemical properties. The repetitions were used for each parameter and each clone.

Determination of soil physiochemical properties

Soil pH was measured using the Potentiometric method (1:2.5 soil:water) (Farooq et al., 2019b). Soil water content was measured by the drying method. Whereas soil bulk density (BD), total porosity (TPOR), and capillary porosity (CPOR) were measured by following Core method of the Nanjing Institute of Soil, Chinese Academy of Science (1978). Total phosphorus (TP) was measured by molybdenum antimony anti colorimetry, whereas available phosphorus (AP) by using the method of Bray and Kurtz (1945). Rapidly available potassium (AK) was measured by Flame atomic spectrophotometry. The total potassium (TK) content was measured by an inductively coupled plasma emission spectrometer. Total carbon (TC) and total N (TN) was measured using a C-N elemental analyzer (Farooq et al., 2021b). An organic carbon analyzer measured the total organic C (TOC). **Table 1** shows the physical and chemical properties of the bulk soil at the test site (**Table 1**).

Quantitative real-time PCR

Nested PCR was used to quantitatively analyze the fluorescence of AMF in roots, rhizosphere soil, and bulk soil. ABI7500 fluorescent quantitative PCR instrument was used for the determination. The process included four parts: primer design of AMF (**Table 2**; Tavasolee et al., 2011), genomic DNA extraction of samples (omega soil DNA Extraction Kit), plasmid cloning (PCR amplification, TA cloning, colony PCR identification positive cloning, Plasmid Extraction) and RealTime PCR sample detection.

TABLE 1 Physical and chemical properties of soil (Mean ± SE).

Determination index	Soil depth	
	0–20 cm	20–40 cm
BD/(g/cm^3)	1.22 ± 0.09	1.24 ± 0.06
TPOR/%	49.53 ± 0.58	49.27 ± 1.04
CPOR/%	37.07 ± 2.23	38.07 ± 1.08
pH	3.65 ± 0.07	3.70 ± 0.06
AP/(mg/kg)	3.76 ± 1.52	2.86 ± 1.75
TP/(g/kg)	0.17 ± 0.01	0.17 ± 0.01
TK/(g/kg)	2.06 ± 0.17	1.98 ± 0.20
AK/(mg/kg)	39.10 ± 3.02a	29.08 ± 1.90b
TC/(g/kg)	12.28 ± 1.44a	6.41 ± 0.77b

Lowercase letters indicate significant differences ($P < 0.05$).

TABLE 2 Primer sequence.

Primer name		Primer sequence
First round amplification	Forward primer	GCATATCAATAAGCGGAGGA
	Reverse primer	GTCGTTTAAAGCCATTACGTC
Second round amplification	Forward primer	TTGAAAGGGAAACGATTGAAGT
	Reverse primer	TACGTCAACATCCTTAACGAA

Illumina MiSeq

Arbuscular mycorrhizal fungi richness was measured in 96 samples of bulk soil, mixed roots, and rhizosphere soil of 15 Chinese fir clones. For microbial diversity detection, fungal AMF was selected. The DNA samples were sent to Beijing ovison Gene Technology Co., Ltd. Paired-end sequencing was used through Illumina miseq PE300 high throughput sequencing platform. AMF first round amplification was primer FLR1 (5′-GCATATCAATAAG CGGAGGA-3′) and FLR2 (5′ GTCGTTTAAAGCCAT TACGTC-3′). Whereas second round amplification was primer FLR3-F (5′-TTGAAAGGGAAACGATTGAAGT-3′) and FLR4-R (5′-TACGTCAACATCCTTAACGAA-3′).

Data analysis

SPSS Statistical Package (SPSS 25.0, Chicago, IL, USA) was used for variance analysis, and QIIME1 (V1.8.0) software was used for α Diversity index analysis (including Shannon and Chao1 indexes). Based on species annotation and relative abundance results, R (V3.6.0) software was used to analyze the histogram of species composition and PCoA. Mothur (V.1.34.4) software for meta stats group difference analysis, Phython (V2.7) software for lefse analysis, Canoco 4.5 software for RDA analysis, and Origin Pro 2021 was used for mapping.

Results

Absolute quantification of arbuscular mycorrhizal fungi

Apart from the S18 clone, the total amount of AMF for all the clones was in the order of rhizosphere soil > root > bulk soil. For the S18 clone, the total AMF amount was higher in roots than in the rhizosphere soil. Overall, the total amount of AMF in root and bulk soil was relatively close (**Figure 2**). However, due to the large variation within the group, no significant difference was observed in the

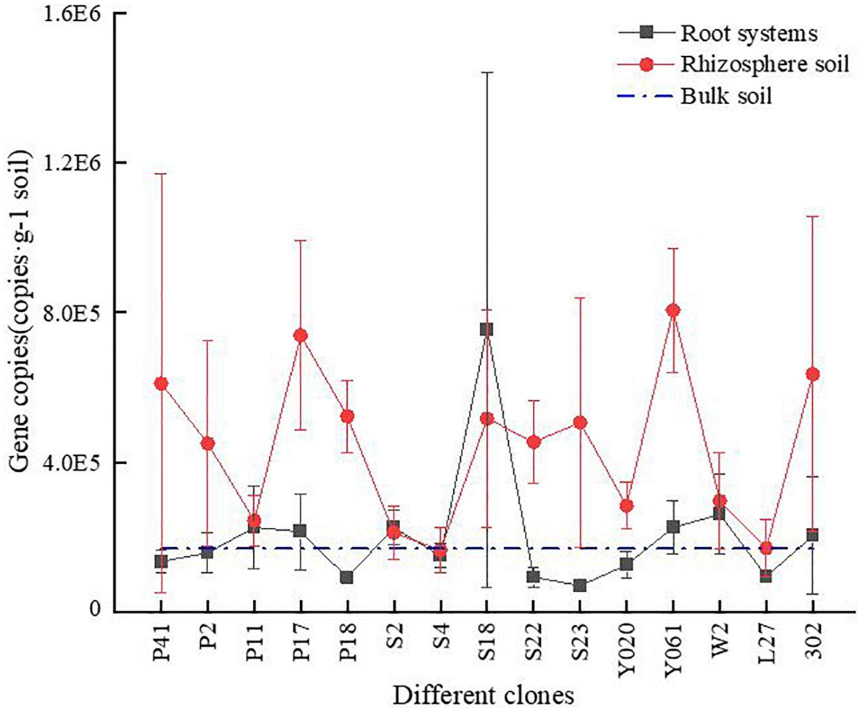

FIGURE 2
The gene copies of AMF in different clones of Chinese fir plantations at different sampling positions. On the horizontal axis, S-NR represents bulk soil, R-represents root system, S-represents rhizosphere soil, and the subsequent number indicates clones.

total amount of AMF between 96 samples of the root system, rhizosphere soil, and bulk soil of all the clones ($P > 0.05$).

Diversity of arbuscular mycorrhizal fungi species

A total of 3,206 OTUs were generated from bulk soil, rhizosphere soil, and root system samples. There were 2,930 OTUs left after leveling. The diversity and uniqueness of AMF species were in the order of root > rhizosphere soil > bulk soil. The AMF composition of rhizosphere soil was similar to that of root and contains most of the AMF composition of bulk soil (**Figure 3**).

The total OTU number and the unique number of roots of Chinese Fir Clone S2 were the highest, 1641 (**Figure 4A**) and 532 (**Figure 4B**), respectively. The maximum number of OTU shared by rhizosphere soil and root system was in clone P17 (923) (**Figure 4C**), and the maximum number of OTU unique to rhizosphere soil was observed in clone P11 (837) (**Figure 4D**). It showed that there were significant differences in AMF flora in different clones. AMF species diversity in Chinese fir clones S2, S23, and P17 were high, and the similarity of AMF composition between rhizosphere soil and root system of clone P17 was the largest.

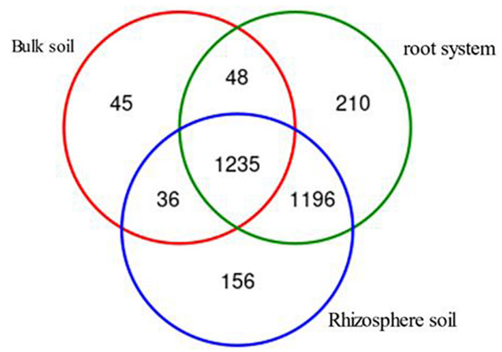

FIGURE 3
Venn diagram of the arbuscular mycorrhizal fungi (AMF) community based on the operational taxonomic units (OTUs) level in different clones of Chinese fir plantations at different sampling positions.

Difference of arbuscular mycorrhizal fungi diversity index among different Chinese fir clones

The change of the α diversity index showed that AMF richness in 15 clone systems was significantly different (**Figure 5**). The Chao1 index of roots of Chinese fir clones S2

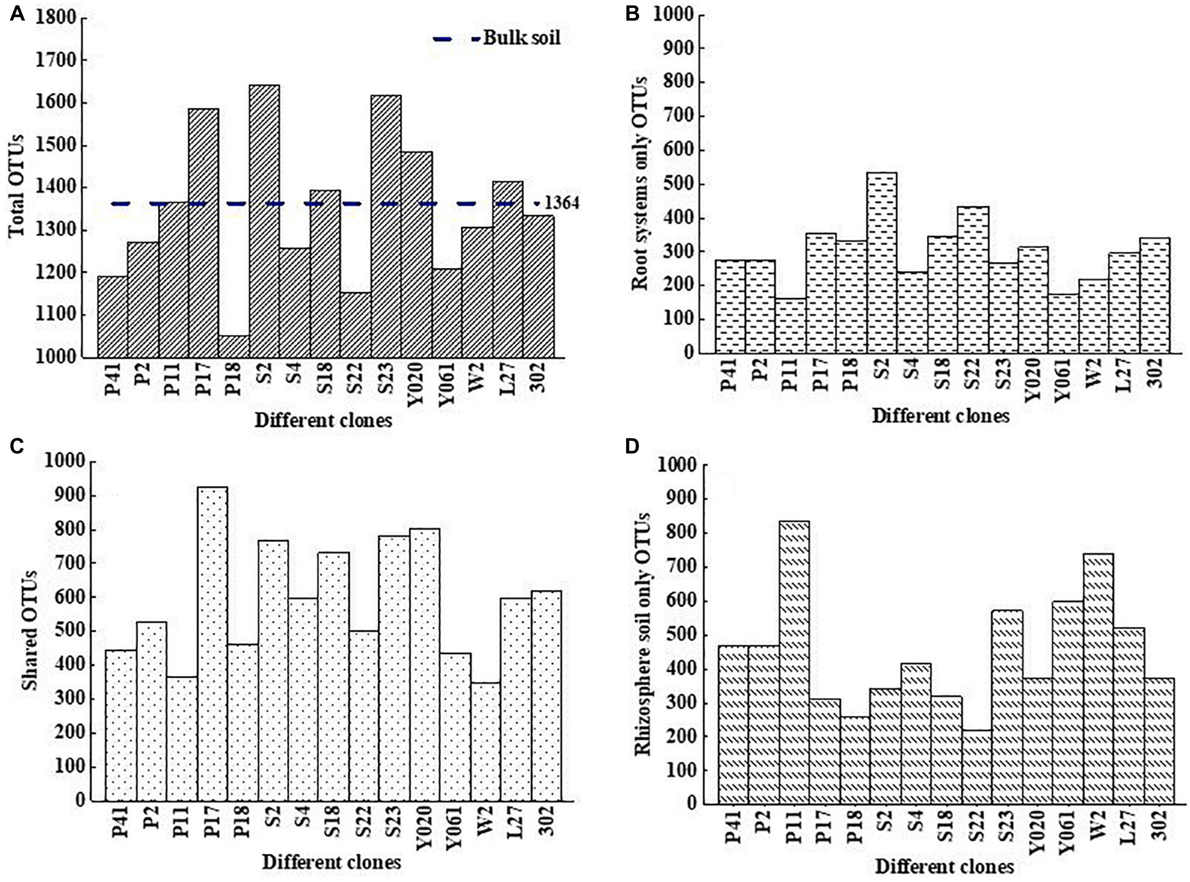

FIGURE 4
Venn diagram of AMF OTUs number in different clones of Chinese fir plantations, the total OTUs **(A)**, root system only OTUs **(B)**, shared OTUs **(C)**, and rhizosphere soil only OTUs **(D)**, respectively. On the horizontal axis, S-NR represents bulk soil, R-represents root system, S-represents rhizosphere soil, and the subsequent number indicates clones.

and P17 were considerably higher while clone P11 was the least, while the Chao1 index of rhizosphere soil of clones S23, P17, Y020, W2, and P11 was significantly higher, whereas S22 clone was the least (Figure 5A). The Shannon index of the roots of Chinese Fir Clone P17 was significantly higher, whereas clones P41 and P11 were the least (Figure 5B), and the Shannon index of its rhizosphere soil was considerably higher than that of clone P18.

Community composition of arbuscular mycorrhizal fungi and inter-group differences

After 96 samples were identified and classified, 2,930 AMF OTUs were obtained, covering 65 species of AMF in 1 phylum, 1 class, 4 orders, 8 families, and 11 genera. *Glomerales* had the highest relative abundance of AMF among all the samples, accounting for more than 99.7%. In addition, there were a certain number of unclassified-Fungi. In terms of the genus, *Glomus* accounted for 90.86%, followed by *Rhizophagus* accounted for 7.71%, *Rhizoglomus* accounted for 1.37%, and *Scutellospora*, 0.03%. Specifically talking about bulk soil, *Glomus* accounts for 89.88%, followed by Rhizophagus accounts for 9.67%. The AMF genus diversity of clone S2 included up to 7 identified and unidentified genera. Other genera and unidentified genera account for very little, and the proportions of different clones were quite different (Figure 6A).

Among the 15 clones, *Glomus* and *Rhizophagus* existed in all samples. *Glomus* accounted for more than 99.7% of the root system of clone S4 and the rhizosphere soil of clone S2. *Rhizophagus* accounted for 29.6% of the rhizosphere soil of clone P18 (the highest). *Rhizoglomus* did not exist in the roots of clone S4 or P17 rhizosphere soil. The 11 AMFs identified at the species level with relative abundances greater than 1% were included in the NCBI nucleoside database (Figure 6B).

Figure 7 showed that *Rhizoglomus* and *Rhizophagus* displayed the most significant contributions to the difference between groups at the genus level for all the Chinese fir-tested clones. AMF dominant genera and species contributed more to

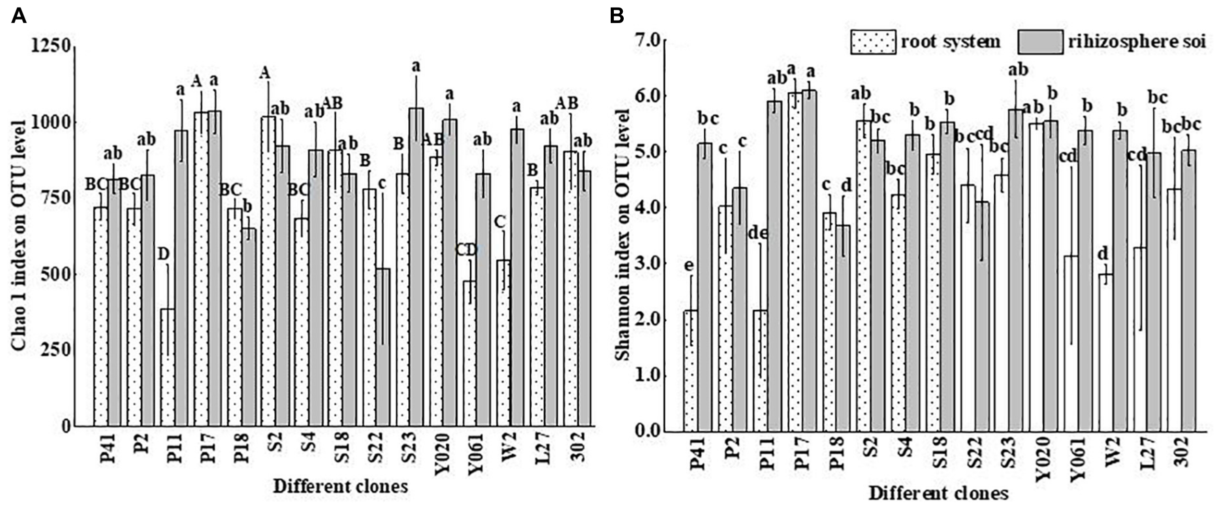

FIGURE 5
The alpha diversity index of AMF in different clones of Chinese fir plantations **(A,B)**. Bars (means ± standard error) accompanied by the same lowercase letter(s) are not significantly different at the 5% probability level. The capital letters in the panel indicate that the difference is very significant ($P < 0.01$), lowercase letters indicate significant differences ($P < 0.05$), the same below. On the horizontal axis, S-NR represents bulk soil, R-represents root system, S-represents rhizosphere soil, and the subsequent number indicates clones.

the difference in AMF community structure of different Chinese fir clones than other genera and species.

Community differences of arbuscular mycorrhizal fungi among different Chinese fir clones

PCoA map showed that in the root AMF community of different clones, the β diversity difference was highly significant ($P = 0.002$; **Figure 8A**). The variation within the clone P11 group was the smallest. The AMF community of the two roots was less affected by external factors, and the degree of overlap between the two was high. The composition and diversity of the two root communities were similar (**Figure 8A**). There was no significant difference in the β diversity of AMF communities in rhizosphere soil of different clones ($P = 0.057$; **Figure 8B**).

Correlation analysis of physiochemical soil properties with arbuscular mycorrhizal fungi diversity index and its relative abundance

Available phosphorus content was significantly positively correlated with the Shannon index, and the TK content was negatively correlated with the Chao1 index. The TP content was negatively correlated with both Shannon index and Chao1 index. AP was significantly negatively correlated with Glomus, and significantly positively correlated with *Rhizophagus*, pH, *Scutellospora*, TPOR and *Rhizoglomus*. There was a significant negative correlation between *Glomus* and *Rhizophagus* (**Figures 9A,B**).

Discussion

Influencing factors of arbuscular mycorrhizal fungi community diversity of different clones of Chinese fir

In general, the diversity of AMF determines plant growth, ecosystem productivity and variability (Van der Heijden et al., 1998; Vogelsang et al., 2006). On the contrary, AMF diversity is also regulated by different plant genotypes (Hart and Reader, 2002). In this study, overall, strong ecological adaptability and rich species diversity were present for AMF; moreover, there was a considerable difference in the physiological characteristics of AMF in different species and genera.

The AMF richness and root β diversity of different clones were significantly different. The AMF species diversity in roots and rhizosphere soil was high for clones P17 and S2. It is probably because they may have more root exudates, attracting different kinds of AMF. On the other hand, they may have better compatibility with multiple species of AMF and benefit from the functional complementarity of multiple AMF species (Koide and Kabir, 2000). The AMF diversity of clones P11 and P41 was low, with a relatively stable AMF β diversity; however, the AMF diversity of rhizosphere soil was high. The reason is that plants will use the supply of C sources to exert

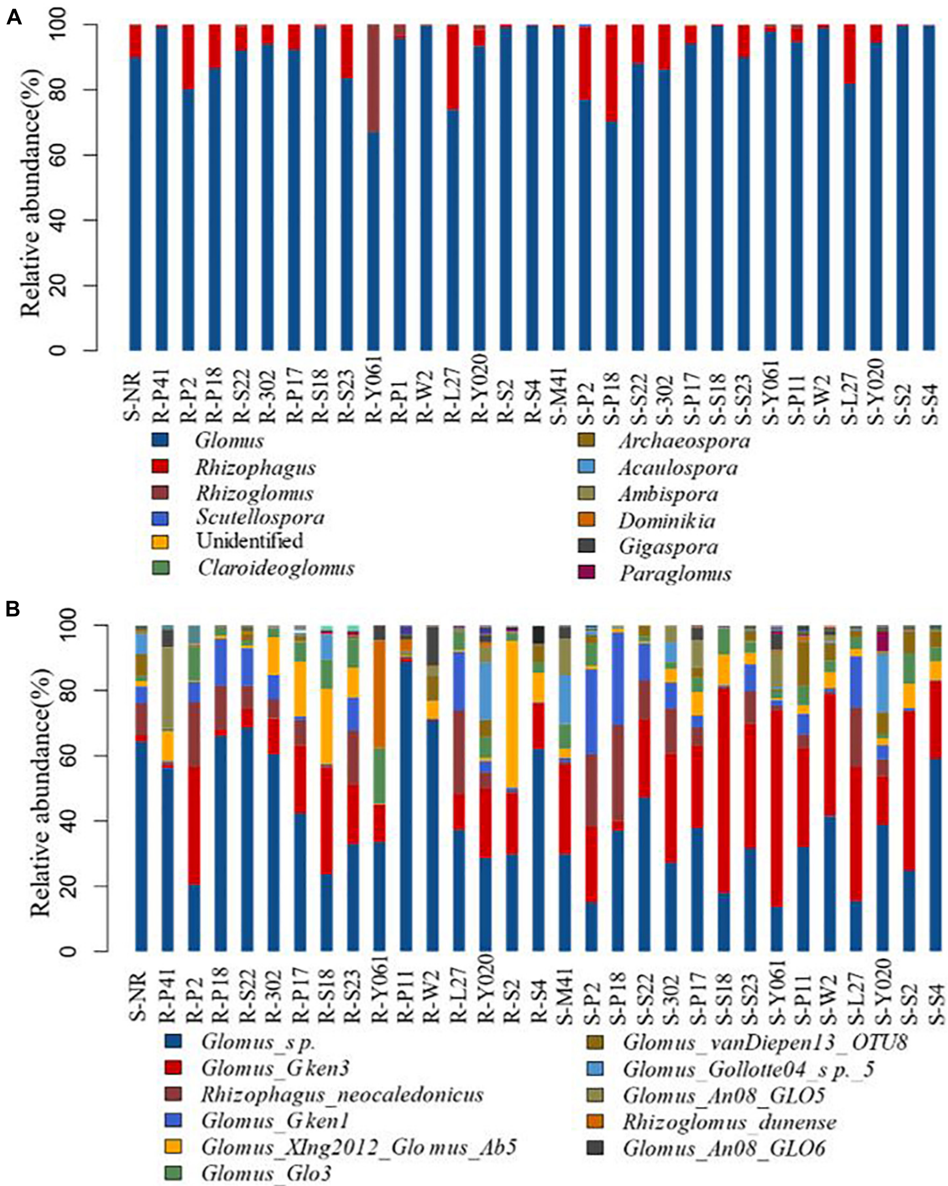

FIGURE 6
The relative abundance at AMF genus level **(A)** and species level **(B)** in different clones of Chinese fir plantations. On the horizontal axis, S-NR represents bulk soil, R-represents root system, S-represents rhizosphere soil, and the subsequent number indicates clones.

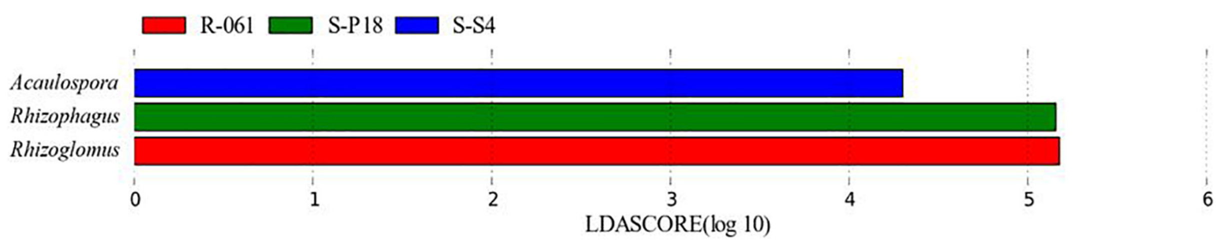

FIGURE 7
LefSe analysis of AMF community.

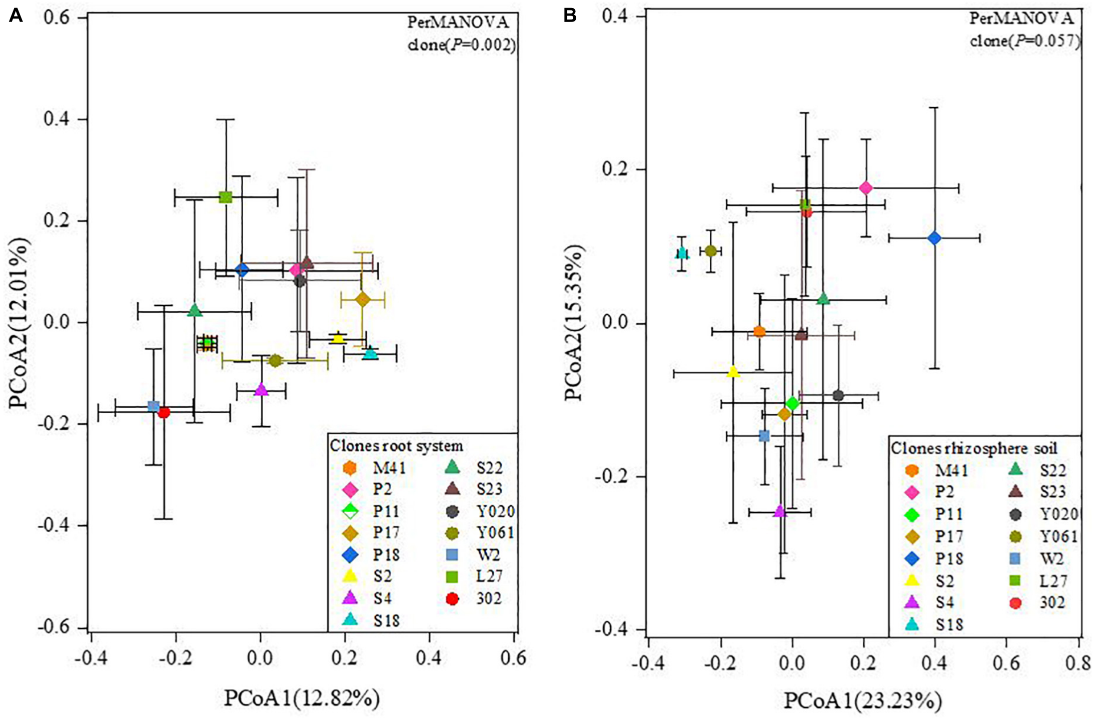

FIGURE 8
PCoA analysis of AMF community in root **(A)** and rhizosphere soil **(B)** of different Chinese fir clones. The numbers in the lower right corner represent the different clones.

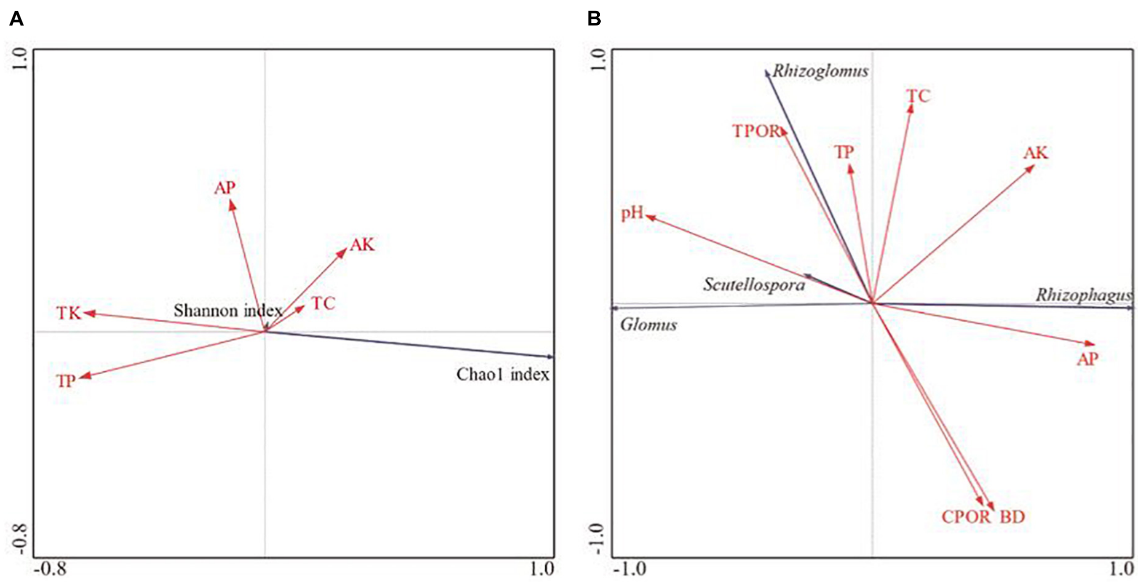

FIGURE 9
Correlation analysis of soil physical and chemical properties with AMF diversity index **(A)** and relative abundance **(B)** in bulk soils.

different selection pressures on different kinds of AMF and prioritize AMF that is more beneficial to themselves (Bever et al., 2009). Different plant genotypes will affect the coexistence of different AMF species in the same root system, and specific plant species will preferentially choose specific AMF species (Alkan et al., 2006; Pivato et al., 2007). This "host preference" makes different AMF C sources or other benefits and affects the AMF community composition of different clones. In the process of plant growth, when the early colonized AMF occasionally replaces the dominant species, the species diversity of AMF will significantly decline (Husband et al., 2002).

Arbuscular mycorrhizal fungi abundance quantifies the impact of Chinese fir clones on it, and it is more sensitive to individual characteristics and genetic diversity of tree species (De Deyn et al., 2011). The AMF abundances of Chinese fir clones S18, Y061 and P17, are high, indicating that these three clones are highly dependent on mycorrhiza and can provide sufficient space and resources for AMF by releasing root exudates to slow down the interspecific competition of AMF, which is conducive to the diffusion and reproduction of AMF. According to the correlation analysis, the relative abundance of *Glomus* and *Rhizophagus* is negatively correlated, indicating that the two AMF species may compete for common resources and space due to their similar niche. As the dominant genus of AMF, Glomus will restrict the propagation and diffusion of *Rhizophagus* and reduce its abundance.

Influence of spatial heterogeneity in the "root system-rhizosphere soil-bulk soil" system on arbuscular mycorrhizal fungi diversity

Arbuscular mycorrhizal fungi diversity is not only regulated by plant genotypes (clones or varieties) but also affected by the soil environment. Under the long-term influence, AMF will evolve into different community compositions (Mirzaei and Moradi, 2017). Some studies have compared natural forests and Chinese fir plantations and concluded that the decrease in soil physical and chemical properties of Chinese fir plantations had caused the diversity changes in the fungal community (Guo et al., 2022). This study shows a significant correlation between AP content and AMF species diversity and its dominant genera. Studies have found that with the increase of AMF species richness, the improvement of plant species diversity and productivity is determined by individual AMF and depends on soil P content (Vogelsang et al., 2006), which further shows that soil P nutrition level is one of the leading factors of AMF diversity. The AMF diversity of rhizosphere soil is higher than that of non-rhizosphere soil. It may be that soil diffusion is less restricted, and AMF taxa with special ecological requirements are also less restricted in the region (Victorino et al., 2021; Zheng et al., 2021). The other reason could be that the rhizosphere is affected by the root exudates of Chinese fir; and the enhanced availability of insoluble nutrients in the rhizosphere changes the interaction between the rhizosphere to facilitate the recruitment and supply of AMF (Zhang et al., 2016).

The diversity of AMF in roots is higher than that in the rhizosphere, which may be because the biomass density of AMF extracellular hyphae in the soil is usually lower than that in the root system, and the distribution of nuclei in extracellular hyphae during spore formation has high genetic variability (Hart and Reader, 2002; Olsson et al., 2010; Deepika and Kothamasi, 2015; Herrmann et al., 2016). It may also be the low saprophytic ability of AMF, which can obtain more C sources in symbiosis with Chinese fir roots, which is conducive to its own growth (Smith and Read, 2008). In addition to the influence of the external environment, different AMFs have different life strategies, resulting in different AMFs appearing in different habitats and obtaining nutrients at different distances/areas from plant roots (Opik et al., 2006; Thonar et al., 2011). Fungal spores may effectively use favorable conditions to form a pool of propagules so that the richness of AMF community in a single plant root or vegetation sample varies with spatial distance and environmental gradient (Li X. L. et al., 2021; Muneer et al., 2022). Therefore, the spatial heterogeneity of the "root-rhizosphere-bulk soil system" has significantly different effects on AMF diversity.

Arbuscular mycorrhizal fungi abundance also has spatial differences, and in the "root-rhizosphere-soil" system, the total amount of AMF is in the order of rhizosphere soil > root > non-rhizosphere soil. It might be because the colonization of AMF in the root system of Chinese fir may be limited by the root space and resources, resulting in a lower abundance in the root system than in the rhizosphere soil (Zhang et al., 2016). On the other hand, the time of AMF infection of Chinese fir seedlings may not be long enough. At the same time, the poor soil environment can limit the survival of AMF, promote AMF to seek nutrients and hosts by using extrarhizosphere hyphae, and thus reduce the abundance of AMF in non-rhizosphere soil (Davison et al., 2015).

Conclusion

By comparing the differences in AMF richness and abundance in the "Root system-Rhizosphere soil-Bulk soil" system of different Chinese fir clones, it was found that the AMF richness and abundance of bulk soil within the same Chinese fir clone plantations were significantly lower than that of root and rhizosphere soil. It indicates that root exudates might activate AMF in the root system and rhizosphere soil, gather in the rhizosphere, and mutually coexist with roots. This study shows *Glomus* is the dominant AMF genus in the infection process among Chinese fir clones. The screening of different Chinese fir clones depicts that clone P17 has high richness and abundance, which may be a nutrient-efficient clone of Chinese fir. Based on the impact of AMF diversity and the difference in symbiosis with different clones, we suggest that AMF diversity can be artificially increased. The screened nutrient-efficient clone (P17) can be planted to coordinate with productivity, resource efficiency and environmental safety of Chinese fir. It will reduce forest management costs and provide a new path for sustainable development of Chinese fir plantation. This laid a foundation for revealing the host genetic regulation mechanism of AMF infecting roots and also provides a reference for the coordinated regulation research and sustainable development of nutrient utilization of Chinese fir in the future.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

PW and YC conceived the idea and designed the experiment. YC, NL, YZ, and JL conducted the study. NL and YC wrote the manuscript. YZ and XM gave suggestions to improve the manuscript. PW reviewed the manuscript and contributed to the discussion. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Author JL was employed by Fujian Shanghang Baisha Forestry Farm.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Impact of land use change on habitat quality and regional biodiversity capacity: Temporal and spatial evolution and prediction analysis

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The ecological stability of a region and the promotion of its coordinated environmental and economic development depend on habitat quality, which is a key indicator of the territory's biodiversity capacity. A case study is done in Suzhou City, Jiangsu Province, to determine how land use changes affect habitat quality. The types of land use in 2030 are simulated based on 2000, 2010, and 2020. The InVEST and CA-Markov models analyze and predict how land use will change in Suzhou. Spatial analysis methods, such as the standard deviation ellipse, the center of gravity analysis, spatial autocorrelation, and random forest models, were used to reveal the spatial and temporal variation characteristics of habitat quality and to analyze its influencing factors. The bare land, building lands, and non-construction lands significantly increased in Suzhou city's land use types between 2000 and 2030 due to land use changes, while the water bodies and forests gradually decreased. Most of the high-quality habitats in this region are found in the water bodies and the mountains. In contrast, the poor habitat quality in this area is mainly concentrated in urban construction lands. The habitat quality gradually declined over time, and its center of gravity followed the migration path from northeast to southwest. The temporal and spatial distribution of habitat degradation in Suzhou reveals a trend of habitat degradation from downtown to suburban areas. This degradation is most common in mountainous and forest areas where the landscape is highly fragmented. Habitat quality in Suzhou city has changed over time and space due to spatial patterns, socioeconomic factors, land use, and the natural environment, with land use having the most significant impact.

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Abbreviations: Invest, Integrated valuation of ecosystem services and trade-offs; HSI, Habitat suitability index; LISA, Local indicators of spatial association; HH, High-High; HL, High-Low; LL, Low-Low; LH, Low-High; GDP, Gross domestic product; GIS, Geographic information system; RS, Remote sensing; GPS, Global positioning system; RSEI, remote sensing ecological index; NDVI, Normalized difference vegetation index; NDWI, Normalized difference water index; SHDI, Shannon's diversity index; FD, Patch density; AI, Aggregation index; CONTAG, Contagion index.

KEYWORDS

land use change, habitat Quality, CA-Markov model, InVEST, ecological environment., ecological stability

1 Introduction

Habitat quality can be defined as the adaptation of an ecosystem for the sustainable survival and development of its individuals and populations, which reflects the regional biodiversity to some extent. It is crucial in maintaining regional ecosystems' stability, the viability of urban development, and human well-being (Hillard et al., 2017). The expansion of construction land and land use changes caused by rapid urbanization reduce urban land use efficiency and then affect the circulation of material flow and energy flow between regional habitat patches, resulting in fragmentation of regional habitat patches, reduction of biodiversity, and ultimately affecting habitat quality (Zheng and Li, 2022). Therefore, evaluating and predicting the quality of urban human settlements is very important.

Early assessments of habitat quality are mainly based on static analysis, focusing on the impact of habitat conditions on species in the specific regional environment. The primary data was obtained through field investigation, which was then summarized into an empirical model, and used to construct an evaluation index system centered on the research species and land use types, such as the riparian vegetation index, riparian quality index (Munné et al., 2003), and river habitat index (Lee et al., 2018; Yang et al., 2018). This method produces accurate results at a high cost, on a limited scale, and with a long turnaround time. With the advancement of information technology, several computerized information models now provide a variety of methods for evaluating habitat quality. Habitat quality research has gradually become more visual, formal, and dynamic and is suitable for multiscale and long-term series (Boumans et al., 2015). Examples include the SOLVES, HSI, and InVEST models (Kubatova and Krocil, 2020; Zhang Y. et al., 2020). The InVEST model is the most comprehensive ecosystem assessment model currently in use.

The original intention of the investment model is to make reasonable decisions in natural resource management. It can quantify ecosystem service functions and show them graphically to determine where investments may improve the welfare of human beings and the environment. The assessment results assist in the management of rational development and utilization of land and other resources. Maintaining a balance between human and natural interests requires the preservation of biodiversity, coordinating environmental protection with economic development, and maintenance. The theory behind the model aims to provide a scientific way for decision-makers to measure the advantages and disadvantages of human activities by simulating changes in the quantity and value of ecosystem services under different land cover scenarios (Nematollahi et al., 2020).

The rapid development of urbanization leads to a rapid increase of bare urban lands and construction land, resulting in an imbalance in land use and a decline in regional habitat quality (Carlson et al., 2019). To understand the distribution patterns of habitat quality, it is necessary to simulate and predict land use change accurately. The automated cellular model, land use change and effects model (Mamanis et al., 2021), system dynamics model (Shen et al., 2012), and Markov model can be used to anticipate and analyze the current land use change (Jon et al., 2021). To optimize land use patterns and regional habitat quality, combining the InVEST and CA-Markov models to simulate the impact of land use change on habitat quality is essential.

Spatial autocorrelation refers to the potential interdependence between various variables affecting the research object within the research area. It is a statistical technique used in geographical research. It is used to check the relationship between geographical variables and the spatial relationship of geographical locations (Diniz-Filho et al., 2009). Moran's I. (Jackson et al., 2010), Geary's C (Yamada, 2021), and Getis are examples of frequent spatial autocorrelations (Pino-Caceres et al., 2022). These methods have both advantages and disadvantages due to their limited applicability. In these methods, the functional space can be roughly divided between global spatial autocorrelation and local spatial autocorrelation. Generally, global spatial autocorrelation is used to describe the global distribution of this phenomenon. The local spatial autocorrelation can be used to identify the range of spatial hot spots and whether or not there is a clear correlation between spatial autocorrelation and special hot spots. If the significance is high, the regional space shows clustering characteristics (Henebry, 1995). Spatial autocorrelation analysis has a long history of research and has been applied in many disciplines, including the natural and social sciences.

As an important city in the Yangtze River Delta urban agglomeration, the ecological restoration of Suzhou is very important for maintaining the stability of the regional ecological pattern. In recent years, Suzhou's economy has been developing rapidly. Rapid urbanization leads to the rapid expansion of urban construction land. Large-scale encroachment into forests and water areas seriously endangers the ecological environment. It is important to understand the advantages and disadvantages of human settlement quality and how it affects high-quality urban development and the regional ecological environment (Zhao et al., 2019; Li L. et al., 2022). However, in the existing literature, limited digital habitat quality research, simulations, and analyses of the spatial and temporal evolution

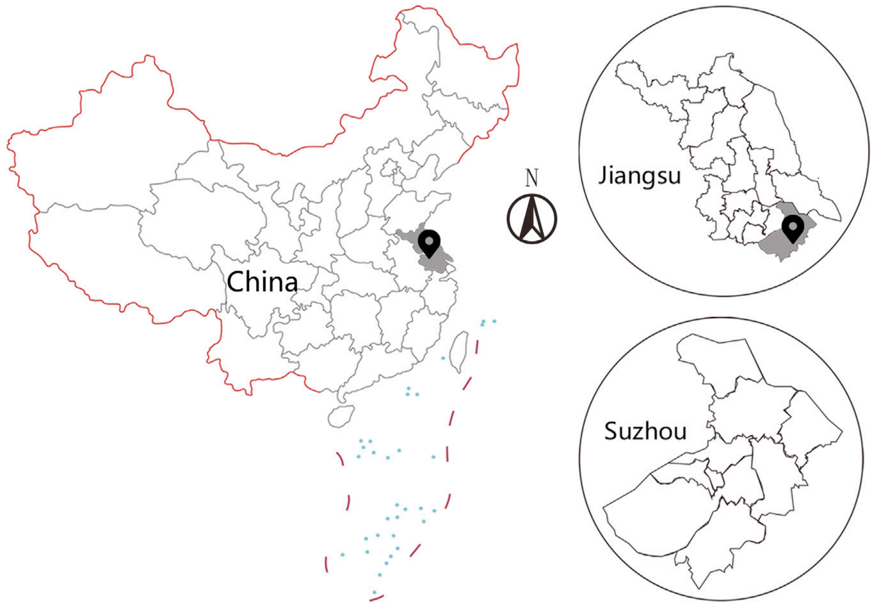

FIGURE 1
Overview of the research area.

characteristics of habitat quality based on land use change's physical and spatial evolution are available. There are few in-depth analyses of the influencing factors of habitat quality from a macro-pattern perspective. This study combines land use simulation with a spatial analysis of habitat quality to observe how it changes over time and which factors will affect it. This approach is very important for the analysis of regional ecosystem services.

2 Material and methods

Suzhou is situated in the southeastern Jiangsu Province of China (Figure 1). It is an important city in the Yangtze River Delta. The area of Suzhou city is 8,488.42 square kilometers (Km). The city is low and flat, with plains accounting for 54.8% of the total area with an average elevation of 4 m. The hilly area accounts for 2.7% of the total land area. The total economic value of Suzhou reached a new height of $ 2 trillion in 2020. Currently, Suzhou is divided into six municipal districts (Gusu District, Industrial Park, Wuzhong District, Wujiang District, High-tech Zone, and Xiangcheng District) and four county-level cities (Taicang, Zhangjiagang, Changshu city, and Kunshan city).

2.1 Research design

Based on the land use data of Suzhou in 2000, 2010, and 2020, the characteristics of urban land use change from 2000 to 2020 were explained by the land use transfer matrix, and the CA-Markov model predicts the land use change in 2000 and 2010. The land use in 2030 was simulated using the 3 years of land use history. The habitat quality module of the InVEST model was used to look at how Suzhou's habitat quality has changed and predict how it will change in the future (Supplementary Figure S1).

2.2 Research data sources

The research data was derived from a raster data set from 2000, 2010, and 2020 with a resolution of 30 m provided by the Resource and Environmental Science Center of China Academy of Sciences (Zhang X. et al., 2022). According to the land use planning of Suzhou, the area is divided into six types: cultivated lands, forests, grasslands, water bodies, construction lands, and bare lands (Figure 2).

2.3 Research methods

2.3.1 InVEST model

When the spatial characteristics of Suzhou City were taken into account, forests, grasslands, water bodies, and other naturally occurring ecological ecosystems were classified as habitats when combined. Whereas the area used for construction was classified as a non-habitat. The precise calculating formula is as follows:

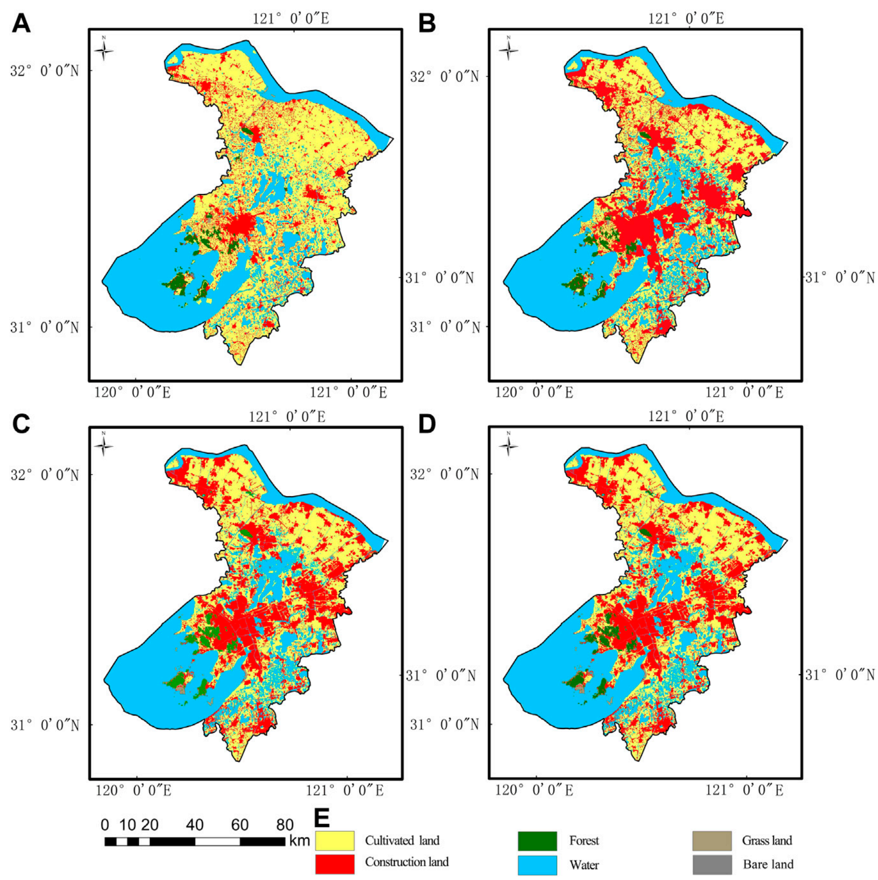

FIGURE 2
Land use types according to the planning strategy of Suzhou **(A)** 2000, **(B)** 2010, **(C)** 2020 and **(D)** spatial distribution simulation of land use type in 2030.

$$Q_{xj} = H_j\left(1 - \frac{D_{xj}^z}{D_{xj}^z + k^z}\right)$$

where Q_{xj} represents the habitat quality of grid x in plot j, H_j represents habitat suitability in plot j, D_{xj} represents habitat degradation degree of x in plot j, K represents half saturation coefficient $D_{xj,}$ and Z represents normalized constant.

$$\mathbf{D_{xj}} = \sum\nolimits_{r=1}^{R}\sum\nolimits_{y=1}^{Y_r}\frac{\mathbf{W_r}}{\sum_{r=1}^{R}\mathbf{Wr}}\mathbf{r_y i_{rxy}\beta_x S_{jr}}$$

where: R and W_r are the numbers of threat sources and the weight of threat source r, Y_r is the grid number of threats, r_y is the stress level of grid X by the stress value of grid Y, and i_{rxy} is the stress effect of stress factor R in Y on habitat grid unit X (Zhao L. et al., 2022).

Generally, executing a habitat quality model requires the influence of distance and weights of stress factors and the compatibility and sensitivity of habitat components to each risk factor. Among these standards, the hazard installation must thoroughly check the impact of land use on regional habitat units. A thorough literature review showed that common hazard sources include cultivated land, urban construction land, unused land, railways, and highways (Li et al., 2022a; Zhang H. et al., 2022; Zheng et al., 2022). It assigns the appropriateness and sensitivity of threat factors according to relevant research. The habitat quality index ranges from 0 to 1, with one indicating medium-to-high habitat quality. The quality of the environment was graded as follows: low (grade 1), medium-low (grade 2), intermediate (grade 3), medium-high (grade 4), and high (grade 5).

2.3.2 CA-Markov model

CA-Markov models are based on soil and land use change resulting from the interaction of several land use types within a region. The land use types represented by the core cell were modified by the land use types represented by the domain cell. The Markov chain controls the time passing through the land-use transformation matrix. Cell automation comprises three parts (Sang et al., 2011; Zhou et al., 2020). 1) Cells: Each cell is a unit containing information about its current state. 2) A lattice forms the cellular space: It is a collection of cells, a generalized two-dimensional cellular automaton permutation. It is triangular, quadrilateral, or hexagonal. 3) Rules: refers to a model of state transition determined by rules, which can be based on the current total of the cells. The state of its domain predicts the subsequent state of the cell. The formula for the expression is as follows:

$$\mathbf{S(t+1) = f[S(t), N]}$$

where S represents the discrete set state of cells, t and t+1 represent distinct times, f represents the fixed conversion rules between cells, and N represents the cell's neighborhood.

In the accuracy test, data from 2000 was used as the baseline, and the above method replicated the land use scenario in 2010. On this basis, the Kappa cross coefficient between the simulated map of 2010 and the actual land use in 2010 was tested using the following contents:

$$\mathbf{Kappa} = (\mathbf{P_o} - \mathbf{P_c}) / (\mathbf{P_p} - \mathbf{P_c})$$

where Po is the proportion of correct simulations, P_c is the proportion of accurate predictions in a random model case, and P_p is the proportion of correct predictions in the ideal case.

2.3.3 Spatial autocorrelation analysis

Spatial autocorrelation describes the important relationship between the attribute value of a specific element in space and its neighboring features, subdivided into global and local autocorrelation. The current research analyzed global auto-correlation. Moran's I was used to determine the spatial quality accumulation, while the LISA index was utilized to determine the spatial agglomeration distribution type of habitat quality. The Get's-Order* was utilized to determine the spatial emphasis pattern of the agglomeration types (Legendre, 1993; Smouse and Peakall, 1999).

2.3.4 Standard deviation ellipse and centerfold shift

A standard deviation is an important tool for studying the characteristics of time and geographical differences. It reflects the degree of spatial and temporal differentiation and spatial agglomeration (Zhang Y. et al., 2022; Zhao Y. et al., 2022). Most of the time, researching the center of gravity is used to figure out the direction and distance of Suzhou habitat quality in the center of gravity's distribution interval over different periods. The equation is:

$$X = \frac{\sum_{i=1}^{n} M_i X_i}{\sum_{i=1}^{n} M_i};\ Y = \frac{\sum_{i=1}^{n} M_i Y_i}{\sum_{i=1}^{n} M_i}$$

Where X and Y represent the longitude and latitude values of the distribution center of Suzhou habitat quality in a particular time interval, X_i and Y_i represent the longitude and latitude values of the distribution of Suzhou habitat quality in a particular time interval, Q represents the habitat quality quantity in a particular time interval, and i represents a particular time interval.

2.3.5 Random forest model

A random forest is an approach for machine learning that uses many trees to train and predict sample data. It builds many models by merging several weak classifiers, assesses the contribution of each variable under various models, and determines the relevance ranking of variables (Xue and Yan, 2022).

2.3.6 Selection of influencing factors

We selected the relevant influencing factors (social economy, land use, and natural environment) from the four landscape patterns (Qi and Wu, 2005; Weber et al., 2018; Clairmont et al., 2021; Zhang X. et al., 2022). As a characteristic of the landscape's spatial structure, landscape patterns represent spatial heterogeneity. The selection of indicators consists of Shannon's diversity index (SHDI), contagion index (CONTACT), aggregation index (AI), and patch density (FD); social and economic activities are significant indicators for measuring the intensity of regional construction and have a significant impact on the regional ecosystem. The selection of indicators comprises population density (PD), per capita GDP, night scene lighting data, and the comprehensive land use index (L); land cover indicators comprise the normalized difference vegetation index (NDVI) and the normalized difference water index (NDWI), and natural environment indicators comprise elevation (EV) and slope (SP) (Supplementary Figure S2).

3 Results

3.1 Land use change

The types of land use in Suzhou are agricultural land, water area, and construction land. Agricultural land accounts for 50.6% of the total area, and water area accounts for 34.9% (Figure 3A). From 2000 to 2010, the land use type with the greatest change was cultivated lands, with a net transfer of 4,357.05 km^2, mainly in water area (274.641 Km^2) and construction lands (1,161.627 Km^2). Secondly, construction land and water area, with 36.708 Km^2 of construction land converted into cultivated land and 70.238 km^2 of water area converted into construction land (Figure 3B). Although the urbanization process is accelerating, the phenomenon of blind reclamation and expansion of cultivated lands and development in Suzhou still exists.

Moreover, the substantial increases in water and construction land areas resulted in a net increase of 274,641 km^2. From 2000 to 2010, Suzhou made great efforts to restore farmland around the lake, and the most obvious achievement was the increase in the

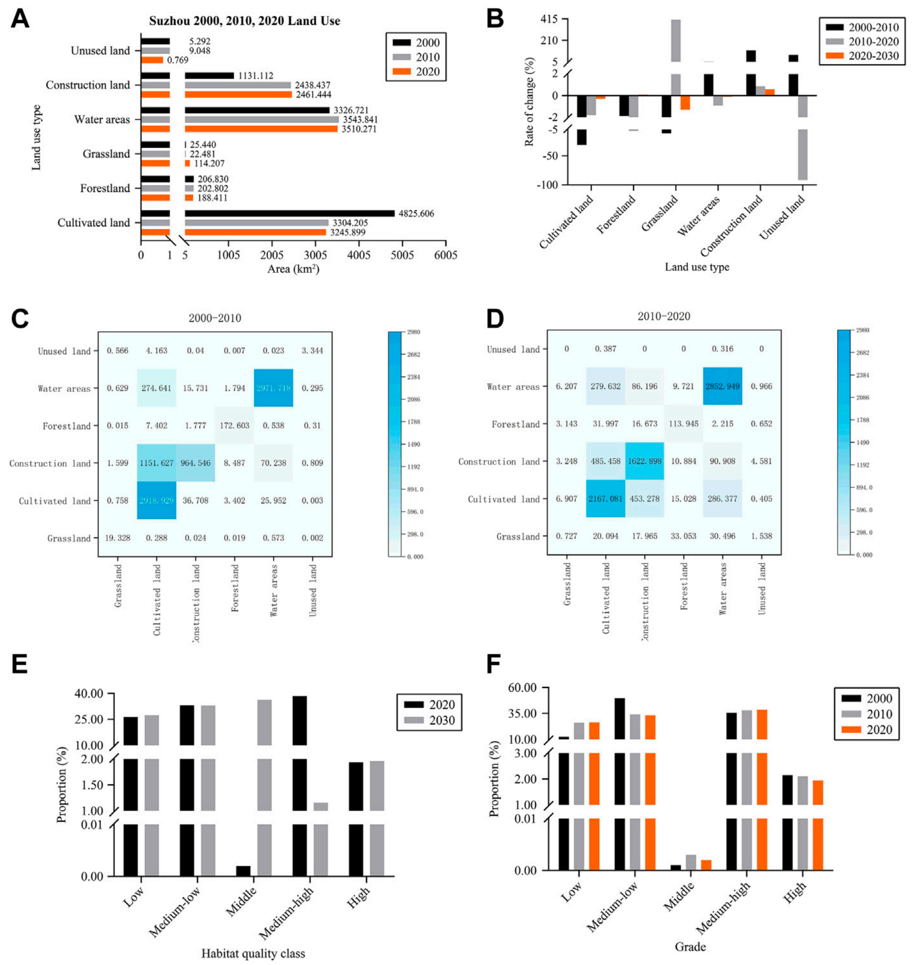

FIGURE 3
(A) Land use type change, **(B)** land use change rate (%), **(C)** 2010–2020 land use transfer, **(D)** 2000–2010 land use transfer, **(E)** habitat quality class, **(F)** Habitat quality grade in Suzhou.

water areas caused by farmland. The net increase in construction land is 1,151.627 km^2, most of which comes from agricultural land, indicating that the increase in urban construction land depends greatly on the continuous invasion of agricultural areas. On the whole, there is little difference between grassland and non-construction area (Figure 3C).

From 2010 to 2020, the biggest change in land use type occurred in water bodies, with a net transfer of 3,263.262 km^2, mainly agricultural land (286,377 km^2) and construction land (90,908 km^2). Rapid urban expansion has increased the demand for agricultural land and urban construction land, eroding urban water systems and lakes. A portion of the second-largest transferred area comprises cultivated and construction land. There has been a net transfer of 2,984.648 Km^2 of cultivated land, mainly 485.485 Km^2 into construction land and 279.632 Km^2 into the water. The net transfer of construction land was 2,197 km^2, mainly including 2 km^2 of cultivated land and 86.196 Km^2 of water transfer (Figure 3D). The forecast of land use types in 2030 based on the CA-Markov model showed that the evolution of main land use types in Suzhou would follow the evolution tracks from 2000 to 2020. By 2030, the areas of unused land, grassland, and water will continue to shrink, while the areas of forests and building lands will expand. The area of forests and grassland increased significantly between 2010 and 2020, while the rate of change of cultivated land and construction lands gradually decreased. Additionally, there was significant pressure on the occupation and compensation of cultivated land. Construction land and gardens experienced the greatest shift among all land uses.

3.2 Habitat quality

3.2.1 Habitat quality in Suzhou from 2000 to 2020

The habitat quality index is related to the ability of the environment to provide suitable habitats for species and

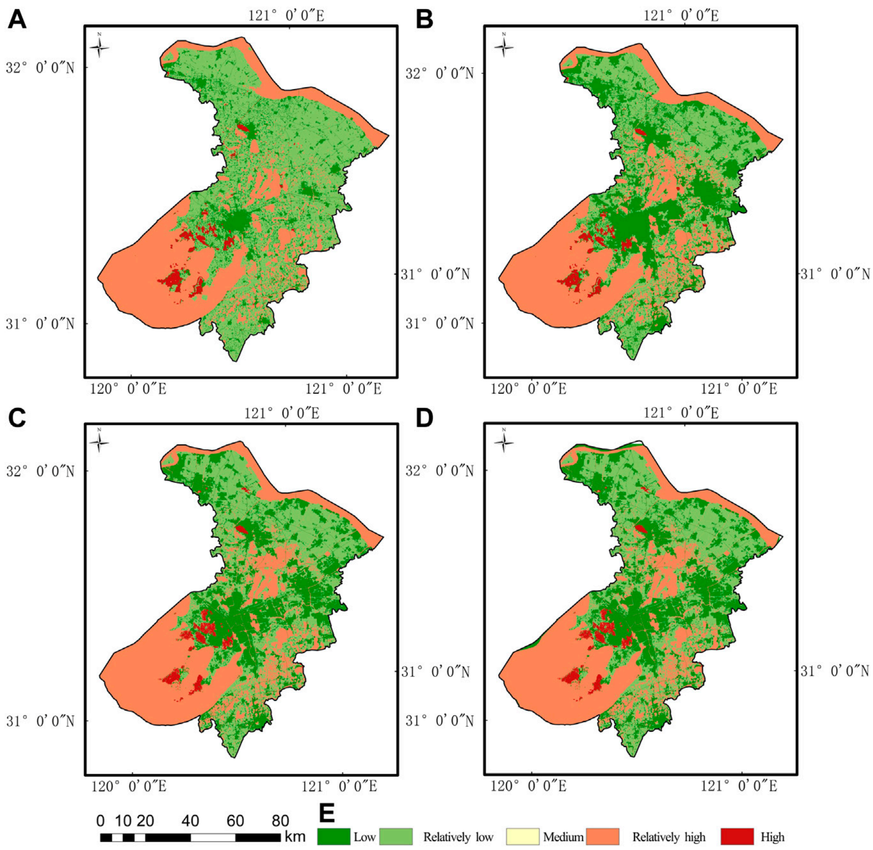

FIGURE 4
Habitat quality and its spatial distribution in Suzhou in different periods **(A)** 2000, **(B)** 2010, **(C)** 2020 and **(D)** spatial distribution simulation of habitat quality in 2030.

maintain population changes. In 2000, 2010, and 2020, the average habitat quality in Suzhou was 0.4656, 0.4168, and 0.4182, respectively; this area showed a general reduction in habitat quality. In 2000, 2010, and 2020, the standard coefficients for the variations in habitat quality were 0.2337, 0.2843, and 0.2867, respectively. The standard deviation coefficient showed an upward trend, indicating the variation of regional habitat quality increased steadily.

Low habitat quality has increased from 2000 to 2010, with an overall increase of 13.44%, to compare the variations in the regional habitat (Figure 3E). Habitats of intermediate and high quality underwent a little change and were relatively stable. Overall, there was a downward trend of high-quality habitat, but the decline was insignificant, accounting for a small proportion. For a long time, inferior and low-quality habitats were the norm, with a low overall balance of high-quality habitats. This indicates that the habitat quality in Suzhou has been very poor for a long time and is continuously declining. From 2000 to 2020, the overall living quality of Suzhou had an irregular spatial distribution, with a low-quality core surrounded by high-quality peripheries. Most high-quality ecosystems were in the lakes and mountains in the south, central, and north of China, including Taihu Lake in the south. There is a lot of forest cover in this area, and the landscape is broken. Therefore, the quality of the habitat has been greatly protected. It is widely distributed, mainly concentrated in big city centers and traffic corridors in different areas, and agricultural land is its predominant land type. Gusu district, industrial parks, and county-level cities in Suzhou have the largest construction areas and the lowest living quality.

3.2.2 Prediction of habitat quality in Suzhou in 2030

The fraction of different habitat quality levels in Suzhou in 2030 was determined using CA-Markov and InVEST models (Figure 4). The changing trend of Suzhou's habitat quality from 2020 to 2030 was consistent from 2010 to 2020, showing an overall downward trend (Table 1). The quality of medium-high-quality ecosystems will be declined by 96.97% between 2020 and 2030. The proportion of high-quality habitats will be increased by

TABLE 1 Change in area and percentage of different habitat qualities in Suzhou from 2020 to 2030.

Habitat quality class	2020		2030		2020–2030	
	Area/km^2	Proportion (%)	Area/km^2	Proportion (%)	Area/km^2	Rate of change/%
Low	2535.65	26.35	2639.65	27.45	103.99	4.1
Medium-low	3195.10	33.20	3175.19	33.02	−19.90	−0.62
Middle	0.251	0.002	3497.92	36.38	3497.67	139.34
Medium-high	3703.56	38.49	112.14	1.16	−3591.42	−96.97
High	187.16	1.94	188.53	1.96	1.367	0.73

0.73%, while the quality of most habitats will be turned to medium and low quality. By 2030, the medium-level habitat area will be increased from 0.251 km^2 in 2020 to 3,497.675 km^2, and this rapid change will be overall.

By 2030, the low-level residential quality in Suzhou will be concentrated in the construction land area, namely Gusu District, Industrial Park, Wuzhong District, Xiangcheng District and High-tech Zone in the central city, Kunshan in the east, Zhangjiagang and Changshu in the north. According to this pattern, the overall living quality of Suzhou will continue to decline through the year 2030. The main reason is that there will be a steady increase in land development between 2020 and 2030, while habitat-friendly land types, such as grasslands and water areas, will gradually decline (Figure 3F).

3.3 Spatial aggregation of habitat quality

The global Moran value and sI values of Suzhou habitat quality from 2000 to 2030 were 0.495, 0.573, 0.5855, and 0.5826 at a confidence level of <0.01, which indicated that Suzhou habitat quality had spatial aggregation. The spatial agglomeration distribution of habitat quality in Suzhou between 2000 and 2030 exhibited the following characteristics: 1) most high-value and high-value agglomeration (HH) regions will be located around Taihu Lake, the northern Yangtze River Basin, and Yangcheng Lake. They are also the main water bodies and lakes concentrated areas in Suzhou. 2) From 2000 to 2030, low-value and low-value agglomeration (LL) regions are primarily distributed in Suzhou's urban core and several county-level cities' urban cores. 3) The LL and HH components have an irregular distribution, and their total proportion is minor. Both high- and low-value agglomeration areas exhibited an upward tendency. However, the total growth rate of the low-value agglomeration area will be slightly higher than that of the high-value agglomeration area, which indicates that the living quality of Suzhou might deteriorate (Figure 5).

3.4 Characteristics of the temporal evolution of habitat quality

In 2000, 2010, and 2020, the center of gravity of the standard deviation ellipse was located in Gusu District, Suzhou. In the past 30 years, the longitude of the center of gravity of Suzhou's habitat quality moved between 120° 58′ 21″ E and 120° 57′ 03.03″ E, while the latitude moved between 30° 41′ 18″ N and 30° 40′ 36″ N (Table 2). The geographical angle of habitat quality increased from 31 00′ 27″ in 2000 to 31 31′ 39″ in 2030. Between 2010 and 2030, the center of gravity of the annual standard deviation ellipse moved 1.992 km to the southwest, 0.035 km to the west, and 0.54 km to the northwest. The migration trajectory of the center of gravity changes into a "C" shape, indicating Suzhou's high habitat quality (Table 3).

The population fluctuation features are notable in terms of spatial distribution, and the entire population is gradually relocating to the Taihu Lake Basin and Lingyan Mountain Area in the southwest. The long axis in each ellipse period is larger than its short axis based on the space length of the standard deviation ellipse, indicating that the direction is quite clear and pointing to the northeast and southwest. Based on the area of the ellipse with three standard deviations, the general trend is "first rising and then falling," indicating that over time, there is an imbalance among the residential quality districts in Suzhou, and the regional spatial agglomeration characteristics are gradually strengthened (Figure 6).

This range represents the degree of habitat degradation caused by existing land-use types. Suzhou's average habitat degradation index from 2000 to 2030 is 0.0259, 0.0424, 0.0382, and 0.0384. The overall habitat quality of Suzhou has deteriorated obviously, and the degradation in a wide range from the periphery to the center is becoming increasingly obvious. The most obvious habitat deterioration occurred in Kunshan, Changshu, and Suzhou industrial park. The city's rapid growth occupies natural places such as forests and wetlands, threatens the urban ecosystem, destroys the landscape, and greatly reduces the habitat quality. Overall, the degree of habitat deterioration in low-level areas is more obvious. Due to the scattered distribution of paddy fields and lakes in the Suzhou area, the habitat environment is more complicated, which leads to the scattered distribution trend of

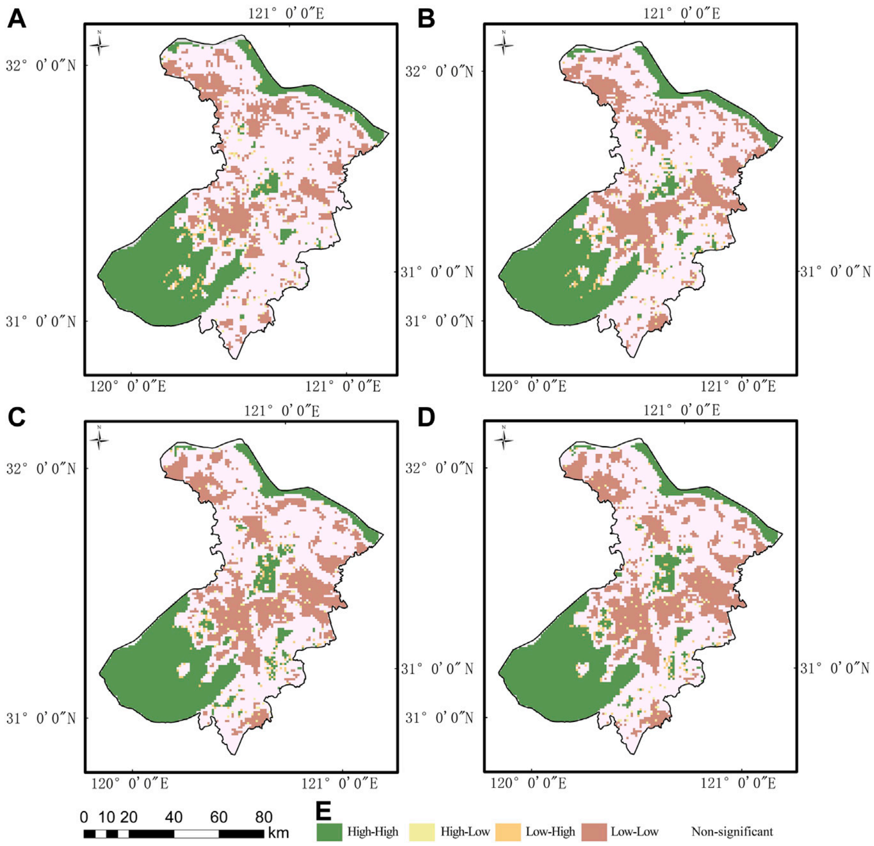

FIGURE 5
Spatial aggregation and distribution of habitat quality in Suzhou in different periods **(A)** 2000, **(B)** 2010, **(C)** 2020 and **(D)** spatial clustering and distribution simulation of habitat quality in Suzhou in 2030.

TABLE 2 Characteristic value of the center of gravity of habitat quality.

Years	Longitude (E)	Latitude (N)	Rotation angle	Acreage	Migration direction	Migration distance
2000	120°58′21″	30°41′18″	31°00′27″	5574.90		—
2010	120°57′25″	30°40′36″	31°33′06″	5639.86	Southwest	1.992
2020	120°57′23″	30°40′36″	31°31′13″	5602.78	West	0.035
2030	120°57′03″	30°40′38″	31°31′39″	5522.45	Northwest	0.54

TABLE 3 The ellipse parameter values for the standard deviation of habitat quality.

Years	Distance along the minor axis	Distance along the long axis	Short/long axis
2000	322.86	549.66	0.587
2010	322.06	557.45	0.578
2020	320.87	555.85	0.577
2030	315.41	557.35	0.566

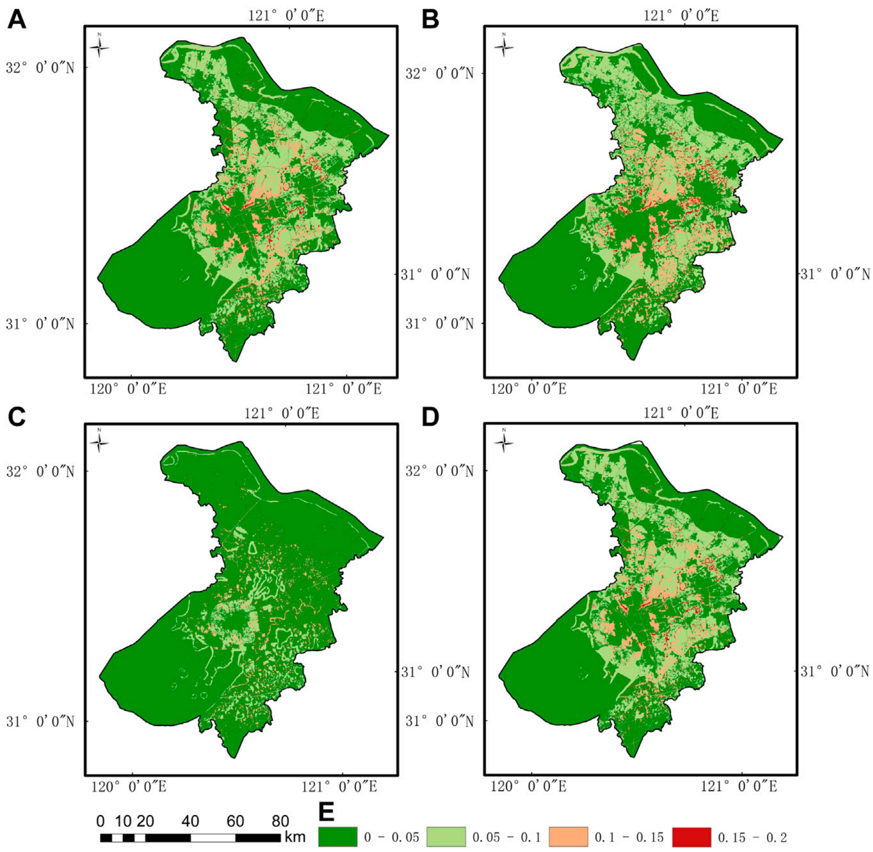

FIGURE 6
Distribution of habitat degradation degree in Suzhou in different periods **(A)** 2000, **(B)** 2010, **(C)** 2020 and **(D)** spatial distribution of habitat quality degradation simulation in Suzhou in 2030.

habitat quality and habitat degradation. The vulnerability of land use categories, such as forests and water, to threats such as construction and transportation land is mostly blamed for this problem. Therefore, the growth of construction land in Suzhou has led to the gradual deterioration of habitats in Kunshan City and Zhangjiagang City, etc., Due to Suzhou's requirement for ecological preservation, the forests surrounding Taihu Lake and Lingyan Mountain are protected, so habitat quality damage is controlled. After utilizing the random forest model, it is found that land use index > NDVI > population density > NDWI > night scene light intensity > elevation > slope > GDP per capital > AI > FD > SHDI > CONTAG are the most important factors for habitat quality (Figure 7).

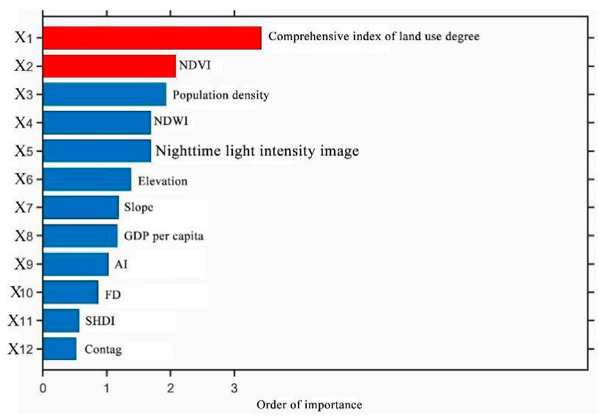

FIGURE 7
Ranking of the importance of influencing factors.

4 Discussion and conclusion

4.1 Causes of land use change

Land use change is greatly influenced by economic development. Economic growth has accelerated the process of urbanization, the industrial structure is close to balance, and the demand for construction land has increased sharply, resulting in a reduction of bare land area. The development of Suzhou's multi-lake fishery has led to an increase in the area of water bodies and a reduction in the area of bare lands. Eventually, the population increase drives urbanization. With the gradual expansion of towns to the surrounding countryside, land use will transform more and more agricultural land into non-agricultural residential and construction land. The economy is in a phase of high-quality development. Urbanization will continue to cause changes, and the cultivated land area will shrink, although the rate of land use change will be slow.

From 2000 to 2020, the spatial distribution of land use in Suzhou showed that the water area and agricultural land were concentrated, while the forest and development land was scattered. Taihu Lake on the southwest, the Yangtze River basin on the north, and several rivers and lakes in the region are the main waters. Agricultural land use is the most common. From 2000 to 2010, Suzhou's construction land expanded rapidly from the center to the periphery, and the water area gradually increased. From 2010 to 2020, the expansion rate of construction lands area gradually decreased, but the degree of patch aggregation increased obviously. In Suzhou, the area of forest, water, and grassland decreased significantly between 2000 and 2030, whereas the area of construction land significantly increased (Lambin et al., 2001).

As an important source of habitat threat in this area, the number of construction lands in Suzhou has increased significantly, particularly in Gusu District, Suzhou Industrial Park, Xiangcheng District, Wuzhong District, and Huqiu District in the center of the city, Kunshan in the east, Zhangjiagang in the north and Changshu in the south. Economic development has an important impact on the change in land use. Economic development has accelerated the process of urbanization, making the industrial structure more balanced, resulting in a substantial increase in the demands for construction lands and a decrease in the bare land area. In addition, fisheries development has led to an increase in water bodies area and a decrease in bare land area. Population growth has led to bare lands turning into other land use types. Numerous socioeconomic and other factors need to be considered to understand what triggers changes in land use (Li et al., 2022b).

4.2 Temporal and spatial variation of habitat quality

From 2000 to 2030, the high HH concentration area was the hottest, mainly concentrated in Suzhou's waters, lakes, and mountain forests. It became increasingly prominent in the center of the Yangcheng Lake area. Suzhou's urban construction land contains most of the coldest places in the city and gradually spreads out from these places. The expansion of cold areas in Suzhou is mainly due to urban construction land expansion. The sharp increase in the distribution of hot spots is partly due to Suzhou's efforts to protect its mountains, forests, and rivers. The main changes in cold and hot spots indicate that the spatial aggregation of habitat quality in the Suzhou area has changed. The areas of the most desirable and coldest places have been significantly expanded, and the spatial collection has improved.

The complete index of land use degrees is the first-factor affecting habitat ranking. Most of Suzhou's cities with high land use intensity values were centered in the area with construction land, whereas the lake and forest regions had low values. The evolution of regional land use is also accelerating along with the growth of urban development. The grassland area has decreased sharply, and construction land is a major threat in this area. This has led to a direct or indirect decline in habitat quality. The surface area also influences changes in habitat quality. In Suzhou, places with a high vegetation coverage index are mainly distributed in mountainous areas such as Lingyan Mountain, Taihu Lake, and Yushan Mountain, and the patches are unevenly distributed. Important wetland regions in the areas with good ecological environment and long-term environmental protection include Yangcheng Lake, the Yangtze River valley on the north side, and many rivers in Wujiang District.

This leads to a high-quality living environment that matches the overall living environment of Suzhou city. The intensity of social and economic activities will directly impact the regional land use and spatial distribution of habitat quality. Low-population-density regions in Suzhou are primarily mountainous and lake areas, whereas construction has minimal influence and low population density. In areas with high light intensity and a vivid night scene, the light intensity in the city's core area is the most concentrated, which increases the demand for development land and encroaches on biological land, resulting in the decline of habitat quality. However, the spatial distribution of Suzhou's GDP per capita is more polarized, with the central city and Kunshan city as the two centers showing a trend toward popularization.

Therefore, it has little influence on regional habitat quality. Altitude and slope are indicators of human architectural tasks accessibility. The city is mainly plains. The terrain is flat, so construction land development is less challenging. Therefore, the habitat quality in mountain areas with higher altitudes and slopes is better than in plain areas. In addition, the landscape pattern index affects habitat quality. Most high-value areas with aggregation, sub-dimensions, diversity, and distribution exist in highly fragmented environments, such as mountains, grasslands, and forests. Low-value areas, such as water bodies, construction sites, and agricultural land, are fairly stable (Martins et al., 2020; Zhang T. et al., 2020; Yohannes et al., 2021). This is related to the spatial distribution of Suzhou habitat degradation.

4.3 Strategies for habitat conservation

For habitat protection and future development in Suzhou, our study suggested establishing a comprehensive habitat quality evaluation system to improve the efficiency of habitat quality management. The ArcGIS Engine is a secondary development platform that allows us to use GIS, RS, and GPS technologies to build an ecological environment quality evaluation model of the RSEI (remote sensing ecological index) model in the visual studio development environment (Kumar et al., 2022). This makes the evaluation work modular and improves overall evaluation. The system can improve the accuracy and efficiency of the evaluation and meet the public's demand for comprehensive treatment and operations of regional habitat quality protection evaluation. RSEI model development, digital information management, and data visualization are effectively integrated into the system, providing technical support for the decision-making management departments. Strictly control land use indicators and accelerate the transformation and upgrading of the land use structure (Xu et al., 2018). Suzhou's land is primarily used for construction and agriculture, but there are few grasslands and woodlands. According to the characteristics of land use and the ecological environment in Suzhou, coordinating land use development and optimizing land use layout are the keys to improving this area's economic and ecological sustainability. Forestry has provided ecological and economic benefits. The artificially planted forest can bring income to Suzhou and improve the natural environment. For the construction of forest land, forest preservation, fire prevention, forest pest management, tree planting, and raising people's awareness of prevention and control are necessary. For bare lands, crop yield is a matter of survival, and higher soil fertility is preferable.

Nonetheless, bare lands are prone to human disturbance. It is necessary to strengthen the protection of bare lands, restrict the occupation of bare lands by construction, and improve the quality of bare lands to increase their resilience against natural disasters. Water may improve the ecological environment and fulfil plant growth needs. Rich resources, such as Suzhou's river systems and reservoirs that can regulate climate and resist drought, have remained virtually unchanged during the research period. Urbanization is rising due to the speed of economic development, which also improves the quality of the ecological environment and sustainably expands biological production. Restricting excessive urbanization's encroachment on forest and agricultural land, enhancing environmental quality, and strictly controlling the growth of building land; also create and expand environmental protection areas (Wang et al., 2022).

The analysis of regional habitat quality showed that the habitat quality in Taihu Lake Basin and the Lingyan Mountain in Suzhou is high. This means that the establishment and expansion of ecosystem protection areas should be strengthened, and ecosystem protection areas should be established in the region's arable land and grassland areas. The ecological core areas, noteworthy areas, environmental protection cache areas, and ecological buffer zones are designated. Taihu lake, Lingyan mountain and many rivers and lakes in Suzhou dominate the core area of the ecological red line and have the highest habitat quality rating. This category should be subjected to the same space restrictions and requirements as non-development areas. All urban development and building activities that conflict with the region's current ecological functions should be forbidden (Yang et al., 2022).

All living and productive activities that invade ecological land must be properly supervised. In addition, it is necessary to strictly manage the transformation of environmental space into urban and agricultural space, infrastructure construction, and the entry of various development projects. The conversion of urban inspection and agricultural production space into ecological space within this controlled region should be promoted if it meets the following criteria. Agricultural land that does not meet these standards should be transformed into ecological agricultural land. Encourage the transformation of ecological red-line regions and buffer zones into core areas. The plan calls for this area's cities, villages, and industrial enterprises to be evacuated orderly. The government should provide resettlement and compensation while formulating strategies to gradually moved the original residents into the controlled area. Encourage people to give up land rights and interests that hurt or destroy the surrounding ecosystem. People can work to restore the ecosystem in places where damage has already been done.

The ecological red line is an important area serves as a buffer zone for various Suzhou parks, forest parks, and natural reserves. Following the requirements of the ecological red line core area, human activities that disrupt or interact with the ecological environment must be tightly regulated. In addition to the necessary protection facilities, all industrial and construction activities that do not meet the main environmental protection objectives in the area should be restricted (Shi and Yu, 2014). Pollution-causing and construction activities, such as releasing sewage, should be strictly regulated. It should be forbidden to transform the control area's ecological space into agricultural production or urban construction. Suppose it is necessary to change land use due to the changes in regional development strategies and the adjustments of higher-level planning; in that case, strict demonstration and approval must be carried out according to relevant procedures.

On the contrary, it should be allowed to transform the controlled areas with a low ecological protection level into coniferous forest areas. It should be strictly forbidden to change the controlled area into an environmental development area or an environmental protection buffer with a low level of protection. The ecological conservation buffer zone should strictly control the scale and intensity of development and construction projects in the area according to the plan and prohibit development and construction activities other than ecological restoration projects, major livelihood projects, eco-tourism projects, and major infrastructure projects. The existing planting industries in the controlled area should be managed strictly with the established laws and regulations. The scale and scope of urban development land or village construction land within the control area must be strictly observed. Any individual or unit should be strictly prohibited from modifying or expanding it without authorization. Meet the conditions of the area to withdraw gradually. For legitimate industrial and mining enterprises in the controlled area, the government should clean up the mining industry according to the requirements of nature reserves and relocate within a time limit (Luo et al., 2021). The transformation from ecological space into agricultural production and urban construction space within the environmental buffer zone should be strictly prohibited. Generally, it should be forbidden to turn ecological conservation buffer zones into ecological compound development zone. Agricultural and urban spaces that meet the conversion conditions should be converted into environmental space. Based on preserving the functional integrity of the ecosystem, the optimization and layout modification of urban and rural land use should be permitted. Intensive and efficient development in the rural industrial area should be encouraged. However, pesticides, fertilizers, and other pollutants must be decreased, and agricultural farming techniques must be encouraged.

4.4 Limitations and improvements

Changes caused by urbanization will continue to reduce bare lands area. Exploring the influencing variables of land use change requires a comprehensive investigation of the influence of numerous socioeconomic and other factors. In our land use change research, the CA-Markov model mimics land use. Regarding the land use parameter setting for the current study, we analyzed elevation, slope, annual precipitation, average annual temperature, population density, and GDP density. The natural environment and socioeconomic data were evaluated for the research's restricted setting but not enough external policy issues.

Consequently, the scientific merit of our research may be strengthened. Several factors affect land use change, including distance and socioeconomic conditions. In particular, land use change in the study area was affected not only by natural processes but also by soils, geography, and other factors, and to a large extent, by human activity, social economy and important government decisions. Therefore, simple meta-automation of linear regression calculation cannot describe the inherent complexity of the interactions of driving factors. The ability to communicate complex nonlinear system relationships between different techniques, such as artificial neural networks and genetic algorithms, has made it possible to determine the relative importance of different factors and create internal transformation rules based on artificial intelligence. The InVEST model was used to analyze the habitat quality of Suzhou, which was characterized by a significant decline in habitat quality, with only a small part of the region showing outstanding habitat quality. However, the InVEST model's parameters were derived by evaluating only pertinent natural and socioeconomic aspects, whereas external macroeconomic policy factors were not sufficiently accounted for. These results can be explained with remote sensing photos, meteorological data, and vegetation information. This is not only a limitation of the current study but also a direction for future research on measuring habitat quality to find out the relationship between evaluating habitat quality based on remote sensing images and measuring habitat quality in field experiments.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

ZL is responsible for writing the articles, the general framework design, and the technical approach. GZ provides theoretical guidance, and ZM provides the proofreading of the articles.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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A sequential game-play modelling on forest-title mortgage loans based on Chinese forester resource and assets valuation

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Forest management has become a critical strategic action because of forests' diverse role in the nature conservation and bio-economic benefits. Forest-title mortgage loan (hereafter abbreviated as "the loan") which is one of forest management methods not only transforms the "sleeping" resources of a forester in her/his forest into assets mortgageable for cash, but also plays a key role in alleviating the shortage of funds that a forester might encounter, promoting financial innovations, and protecting forest resources. As such, this paper examines the problem of the low limit placed on this loan in China, and draws the following conclusion from employing the dynamic game method comprising complete information: in the actual mortgage market where banks hold an absolute advantage in issuing mortgage loans to the borrower (whether or not a forester acting as the borrower here applies for this loan from the bank through using an asset-appraisal agency) the amount of loan approved for the forester is going to be lower than the actual market value of the forest-resource assets that the forester owns. At the same time, based on the above conclusion, this paper proposes certain suggestions regarding how to raise the limit of this loan for the forester. These suggestions include proposals pertinent to the governmental policy support, introduction of innovative credit products developed by the banks, as well as the elaboration on how foresters could integrate their forest-resource assets by using cooperatives and launching scaled productions.

KEYWORDS

forest management, forest-title mortgage loan, game theory, asset appraisal, China

Introduction

Due to forests' diversified roles in nature conservation and bio-economic effect that enhances social, ecological, and economic benefits, forest management has become a critical strategic action (Farooq et al., 2019, 2021; Zou et al., 2022). Forest-title mortgage loan which is one of forest management methods not only transforms the "sleeping" resources of a forester in her/his forest into assets mortgageable for cash, but also plays a key role in alleviating the shortage of funds that a forester might encounter, promoting financial innovations, and protecting forest resources. The forest-title mortgage loan refers to a type of a loan which a borrower takes out from a financial institution using her/his ownership of, or the right to use her/his forest or forestland as the collateral for repaying the loan. Its innovational aspect lies in the fact that it has broken up the single pattern formed long ago in which a bank uses a borrower's real estate(s) as the collateral(s) for the loan. Hence, this type of loan has transformed the resources previously sleeping in the forester's forest into assets mortgageable for cash. Zhou and Li (2014) believe that forest-title certificates exist only in China; all other countries adopt the small mortgage loan scheme in their rural areas. From a global perspective, most developed countries are highly experienced in operating forestry financing. For example, the U.S. provides special, low-interest microloans to their mini foresters. At the same time, it subsidizes forestry practitioners through fiscal aid to compensate for the risk and loss incurred to them due to forestry's long growth period (Metodi et al., 2020). France also provides long-term and low-interest forestry loans to its foresters with a term of over 20 years and an annual interest rate of less than 3%. As for Japan, it even provides long-term and interest-free loans to its forestry operators. Even among the developing countries, there are also cases where some of them explore and implement the eco-forest loan system. For example, in Costa Rica, there is a Nectandra Institute, a small non-profit organization, which has launched a zero-interest eco-forest loan program. Abbreviated as ELF (Eco-loan Financing), this program aims to support local forest conservation and restoration efforts (Lennette et al., 2011). In addition, based on the recommendations of the United Nations Conference on Environment and Development and Ministerial Conference on the Protection of Forests in Europe, Turkey began preparing for its National Forestry Programme (NFP) in 2001 (Canan and Koray, 2022).

In 2013, the Chinese government formally approved and enacted a series of policies regarding the operation of forest-title mortgage loans and standardized their disbursement system, the measure of which enabled this loan to develop initially in China. The disbursement of this loan to the foresters plays a key role in alleviating the shortage of funds that these foresters might have, promoting financial innovations, and protecting forest resources. However, China is at a preliminary stage of exploring this loan system with certain problems inevitably arising in the course of its implementation process. As such, this paper looks into the problem of low limit placed on this type of loan in China and figure out some solution. We draw the following conclusion through use of dynamic game methods embodying complete information: in the real market where banks hold an absolute advantage in issuing mortgage loans (whether or not foresters apply for this loan through asset-appraisal agencies from the bank), the amount of loan that they get is going to be lower than the actual market value of their forest-resource assets. Furthermore, based on our results of "Gameplay process between the forester and bank" and "Game process among forester, bank, and asset-appraisal intermediary," the paper also proposes some suggestions regarding how to raise the limit of this loan for the foresters, including elaborating on relevant governmental policies, innovation of credit-related products produced by the banks. Moreover, it provides some suggestions of how to have foresters integrate her/his forest-resource assets and launch scaled productions through cooperatives. This study is significant for solving the problem of low limit placed on this type of loan and some improvement in forestry management in China.

The rest of this paper is organized as follows: Chapter 2 is literature review, Chapter 3 presents gameplay process between the forester and bank, Chapter 4 presents the game process among forester, bank, and asset-appraisal intermediary, Chapter 5 provides countermeasures and suggestions, Chapter 6 is the conclusion.

Literature review

When scholars study agricultural loans, most of them evaluate the loans from the perspective of banks. Bryant (2001) developed an Agricultural Loan Evaluation Professional System (ALEES) to help banks and other financial institutions to evaluate the agricultural loan applications they received. Bruce and Hagan (1973) believed that in order to determine the current quality of loans and assess the current financial status of each borrower, agricultural lending institutions were faced with the long-term task of regularly assessing the personal and financial attributes of borrowers and proposing new credit-scoring items for them.

China has uniquely adopted the forest-title mortgage loan system making it possible to turn the "sleeping" assets of forest resources into cash, which could not only effectively protect the forest resources, but would also alleviate the financing difficulty that foresters may be having to an extent. It could help them acquire wealth without them cutting down the trees they own, hence it would promote rapid rural economic development. Since 2003, China has operated under the forest-ownership mortgage mode (Liu et al., 2020). This mode refers to the loan system in which the lending institution uses certificates of ownership of forests or forestlands from the enterprise or forester as collateral for the loan. Around 2013, this mode was empirically confirmed to be in line with the Chinese market mechanism and subsequently gradually improved. However, the operational mode of this loan geared towards the foresters only covers a low loan amount and was subject to further improvement (Zhou and Li, 2014). This loan has opened up a new business operational field for the financial institutions, improved their operational efficiency, and fully realized the "triple-win" of forestry, foresters, and banks. However, this loan amid the foresters is still during its exploratory phase in China. As it is being implemented, there has emerged problems of foresters unable to get their desired amount of loan. In other words, it is difficult for the foresters to meet the financing needs they are having through taking out this loan (Ding and Zhang, 2012; Xie et al., 2014; Zou, 2020). The approval of loan applications filed by the foresters may face many restrictions (Lu et al., 2018). For example, commercial banks may impose some sort of restriction on it since the term of the loan is shorter than the period of production and operation of the forestry of the forester, or the forester's mortgage ratio of the loan is too low (Yu and Liu, 2011). At the same time, some common problems exist over this loan: low approved amount, short repayment terms, and high interest rates, to name only a few (Xiao and Fan, 2011; Jiang and Yu, 2019). Scholars have done a lot of research with respect to the causes for such problems. Most scholars believe that the great risk associated with the financing process of this loan is what restricts the financing development of it. Forest resources have some special traits. For example, they have a long growth cycle and are easily impacted by natural disasters. These special traits make up the important reason for this type of loan to face high financing risk (Duan et al., 2021). Ochoa-gaona (2001) and Chen and Innes (2013) pointed out that forest resource assets are often operated with the backward technology, they are poorly managed, and have certain inherent limitations. These characteristics of the forest resource assets usually lead to the foresters bearing high risks while managing them. Thence, it is difficult for the foresters to get financed using such assets as collateral. Zhou and Li (2014) and Duan et al. (2022) pointed out that forest resource assets are characterized by their long production cycle, slow investment return, slow capital turnover, and vulnerability to natural disasters, which restrict the supply of forest-related funds by banks and other financial institutions, at least to some extent. In addition, many scholars believe that the lack of the effective evaluation on the collateral with forest titles, which is the forest-resource assets in effect, is an important reason behind the low approved loan amount among other difficulties (Li and Chen, 2021). Wu (2018) pointed out that the asset value evaluation system of forest titles has not yet been established, so it is difficult to fairly and rationally assess the value of forest-title resource assets being mortgaged. Cai and Zeng (2011) and Wang et al. (2014) believed that the difficulty in assessing the forest-title resource assets being mortgaged and the high risk involved in disposing of them were the important factors leading to the low loan amount being approved of. At the same time, related supporting measures such as forest resource asset appraisal are not yet mature, and there is a lack of erection of professional forest-asset appraisal institutions. As a result, the assessed value often deviates from the actual value of forest-resource assets, resulting in a small loan amount being disbursed to the borrowers (Lei, 2020; Ma et al., 2021). Therefore, in the light of the above problems, many scholars put forward some suggestions regarding how to optimize the valuation of forest-title assets. Qiu et al. (2018) and Xie and Su (2020) argue that the asset-appraisal intermediary makes up an important part of the evaluation system correctly assessing the value of forest-title assets. Strengthening the construction of such intermediaries is beneficial towards improving this system. Hence, it can reduce the deviation between the real value of assets and their assessed value, and guarantee the proper loan amount. At the same time, Xu (2018) and Xie (2019) proposed that forest-resource asset-appraisal intermediaries should not only increase in number, but also strengthen their professional and market-oriented construction, and foster a group of high-quality forest-resource asset appraisers. Through professional evaluations performed by the forest-resource asset appraisers, the value of forest-resource assets is determined scientifically to ensure the accuracy of such evaluations.

The above research results on the forest-title mortgage loan provide materials and experience for us to understand the current situation under which this type of loan operates in China. From the perspective of research content, it focuses on the loan difficulty and low loan-limit existing in the forest-title mortgage loans and the reasons behind the formation of these problems. At the same time, we propose that forest-resource assets should be evaluated by intermediaries in charge of appraising the respective forest-resource assets to ensure that the loan quota of the mortgaged property is equivalent to the actual value of the forest-resource assets. Therefore, based on the previous research results of many scholars, this paper studies the loan quota of foresters in the absence and presence of asset-appraisal intermediaries. Our research shows that in either case, the loan amount that foresters get through the mortgaging of their forest-title assets is lower than the actual market value of such assets.

With respect to the method of research, this paper adopts the dynamic game-playing method embodying complete information. It studies the issue of forest-title mortgage loan limit reflecting the forest-resource's asset value that exist between the foresters and the banks, or among the foresters, banks, and intermediary asset-evaluation agencies. In addition, it sorts out the application of game theory towards the forest-title mortgage loans. Most scholars use the game theory to study the behavior of both lenders and borrowers within this framework. Jin (2017a) used the static game model embodying complete information to find the fact that financial institutions could not fully grasp the effective information of foresters' management of forest-resource assets. In order to reduce the adverse selection and moral hazard caused by the information asymmetry, credit-rationing measures would be adopted to guarantee the credit security, leading to the difficulty in ensuring the availability of credit for foresters. In the meantime, Jin (2017b) used dynamic and repeated game analysis to conclude that financial institutions should adopt credit contracts to reduce credit risks to foresters or other credit seekers. Zhang and Zhang (2015) applied evolutionary games and imperfect information motion, respectively. The availability and the interest rate of forest-title mortgage loans are analyzed by the state game. The results show that the most important factor affecting the availability of loan is the realization ability of forest title, and strengthening the effectiveness of guarantee of forest-title can improve the availability of loan. Meanwhile, the interest rate of the loan is directly proportional to the degree of information asymmetry between the lenders and borrowers. Liu and Yang (2011) used game theory to simulate the objective situation and behavior of both lenders and borrowers. They studied the risks of default and other influencing factors faced by financial institutions in issuing forest-title mortgage loans. The results show that the loan amount and interest rate are in direct proportion to the risk of default, but if the financial institutions increase the penalty for borrowers to get defaulted of their loans, it could reduce their risk of default. Liao et al. (2011) drew the conclusion by setting up the game behavior model among foresters, forestry enterprises, and financial institutions that loan interest, mortgage value, and credit mechanism are the important factors affecting the behaviors of both lenders and borrowers of the forest-title mortgage loan. Deng et al. (2022) constructed an internal financing model that the purchaser acts as the core leading enterprise to provide loans when the farmer has fixed assets as collateral. The result shows that fixed assets would increase the expected profit of the farmer and redistribute risk and profit between the purchaser and the farmer. Zheng and Xie (2021) found out through analyzing the process of gameplay by banks and foresters regarding forestry loans that banks were more willing to issue loans to foresters with high credit. When faced with an imperfect credit system in the market where low-credit foresters are situated everywhere, banks would choose not to grant loans to such foresters. This would influence the development of the whole loan market. Chen et al. (2021) analyzed the mechanism of cooperative-guarantee mode on forest-title mortgage loan by establishing a two-stage game model involving cooperatives, foresters, and financial institutions. Results show that the cooperatives' support for loans to foresters has a positive effect on the demand for this loan. The increase in penalties to foresters for defaulting the loan has a positive influence on guaranteeing the loan's repayment rate during the repayment period, but has a negative influence on the demand of loans made by the foresters. The study also shows that the cooperative assumes too much responsibility for repaying the loan, which is not conducive to the overall social utility of this loan. At the same time, a few scholars used the game theory to study the behaviors of governments and financial institutions with respect to this loan. Wang et al. (2021) through constructing the model of gameplay by local governments and financial institutions, analyzed the evolutionary process of this behavior. The result shows that policies implemented by local governments in support of issuance of this loan is necessary and feasible towards compelling the commercial banks to launch business pertinent to this loan. Some latest studies illustrate that the government should play a significant role in the operation of Forest-title mortgage loans.

The above research results on applications of the game methods provide materials and experience which we can understand in the context of forest-title mortgage loan. However, from the perspective of the research content, most of them are limited to the game of different behaviors between the lender and borrower of the loan. Although there are also discussions regarding the game of interests of all parties in the loan, such as the loan's interest rate, mortgage value, and risk of default, in general there are almost no research results on the game of interest between lenders and borrowers, as well as the asset-appraisal intermediaries of the loan. Therefore, based on the idea of social welfare maximization and Pareto optimum, this paper applies the method of dynamic game comprising complete information to provide a theoretical explanation of the reasonable pricing of forest-resource assets, which is equivalent to the loan amount, between the lenders and borrowers and between the lenders and asset appraisal intermediaries of this loan. The equilibrium solution of social welfare and individual surplus maximization and Pareto optimum is obtained by deducing the game model to explain the phenomenon as to why the amount of this loan is generally low in reality.

Gameplay process between the forester and bank

We assume that there are two players in the market of borrowing and lending of forest-title mortgage loan: the forester and the bank. They play a game on the evaluation of assets of forest resources owned by the forester, which directly affects the amount of loan to be approved of by the bank. We suggest that there are two situations of gameplay process between the forester and bank. One is the forester and bank are equally powerful in the mortgage market, and the other is banks are more powerful in the mortgage market. We do not believe that the forester has more powerful in the mortgage market, because forester has limited channels to obtain mortgage loans but bank has many different types of customers.

The forester and bank are equally powerful in the mortgage market

As the information on forest-title mortgage loans is fully public in the market, we build dynamic game models comprising complete information that based on the idea of social welfare maximization and Pareto optimum to provide a theoretical explanation of the reasonable pricing of forest-resource assets. In the situation where both parties are equally powerful in the market, the bank will be the first to quote the value of assets of forest resources, which is equivalent to the amount of loan that the bank is willing to grant to the forester. In other words, the bank will give an initial quotation of P_1. If the forester is dissatisfied with the bank's quotation of P_1, he gives a counteroffer. If the bank is not satisfied with the forester's counteroffer, the bank will give a new quote. In the case of the forester who is unsatisfied with the

FIGURE 1
Quotation and reserve price.

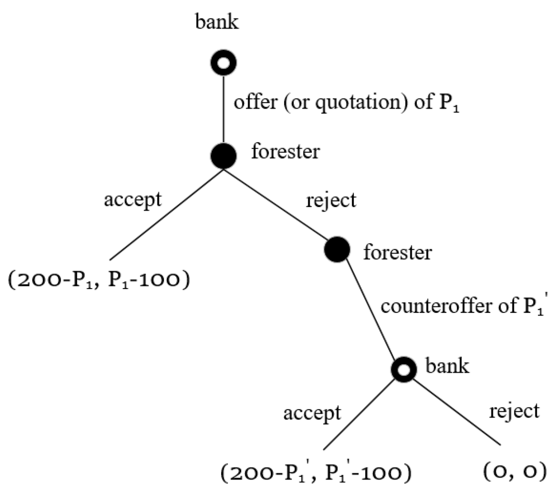

FIGURE 2
Game tree.

bank's new quote, she/he will make a new counteroffer again, and so on. In the bargaining process of the two parties in question, there is a reserve price of P_b to the bank. When the negotiated price is higher than this reserve price of P_b of the bank, the bank would rather choose not to grant the loan to the forester. On the other hand, the forester also has a reserve price of P_f. When the negotiated price is lower than this reserve price of P_f of the forester, the forester would rather not take out this loan from the bank. Figure 1 shows the relationship between the offered and reserved prices obtained by both parties of the game according to their judgment of the value of assets owned by the forester. Among them, P_f and P_b are the reserve prices of the forester and the bank on the loan amount, respectively. P_1 and P_2 represent the initial and second quotations of the bank regarding the approved loan amount, respectively. P_1' and P_2' are the first and second counteroffers of the forester on her/his desired loan amount, respectively. It can be seen from Figure 1 that only when the reserve price of the forester is less than that of the bank can both parties bargain successfully. The range between the reserve price of the forester and that of the bank is called the agreement-reaching range.

In the "bargaining game" played between the forester and bank regarding the amount of loan to be granted by the latter to the former, if we assume that the reserve price of the forester to be 100 and that of the bank to be 200, then [100,200] would be the range where an amount agreed upon by both sides may appear. As the transaction price P gets closer to 100, the bank's surplus is larger, and the forester's surplus is smaller. As the transaction price P gets closer to 200, the forester's surplus is larger, and the bank's surplus is smaller. Thus, the bank's surplus can be expressed as 200-P, while the forester's surplus can be expressed as P-100.

Considering the characteristics of a "bargaining game" played by the two parties in question, we could use a dynamic game consisting of complete information to explain this game process: the bank takes the lead in giving the value that it considers equal to that of the forest-resource assets, which is the price P_1, or the actual loan amount, that the bank is willing to pay. If the forester accepts this initial offer from the bank, the game ends, and the forester can borrow this amount from the bank according to the offer P_1 of the bank. In this case, the bank's surplus is 200-P_1, and the forester's surplus is P_1-100. If the forester does not agree with this initial offer of the bank, she/he will make another offer, which is P_1'. At that point, the bank has two choices to make: accept or reject it. If the bank accepts the forester's offer, the bank will grant the amount to the forester according to the forester's offer, and the game ends. In this case, the bank's surplus is 200- P_1', and the forester's is P_1' -100. If the bank rejects the forester's offer P_1', then the game is over, and the result is that the loan transaction fails, and both sides of the game get zero return. This game process is shown in Figure 2.

The game shown in Figure 2 is a dynamic game consisting of complete information. We could solve for this game's subgame by using "backward induction" and refine it as Nash equilibrium. In the last round of the game, if the bank accepts the forester's offer P_1', then the bank's surplus is 200- P_1'; if the bank does not accept the forester's offer, then it is surplus is 0. Thus, when 200- $P_1' > 0$, or $P_1' < 200$, the bank will accept the forester's offer. A rational forester would set his offered price as $P_1' = 200 - \varepsilon$, where ε is an arbitrarily small positive number, so as to maximize her/his surplus. The forester's surplus is $100 - \varepsilon$, and the bank's is ε. Therefore, the game tree previously shown in Figure 2 has now become the one shown in Figure 3.

In the game shown in Figure 3, if the forester accepts the initial offer of P_1 from the bank as the loan amount, the forester's surplus is $P_1 - 100$. If the forester rejects the bank's initial offer of P_1, her/his surplus will be reduced to $100 - \varepsilon$. Therefore, if the initial offer P_1 of the loan amount of the bank meets the following formula, then the forester will accept the bank's offer, and the game ends:

$$P_1 - 100 \succ 100 - \varepsilon, or P_1 \succ 200 - \varepsilon$$

That is to say, if the bank's initial offer of the loan of P_1 is close enough to 200, the forester will consider accepting the bank's offer, and the game ends. If the bank's initial offer is not close enough to 200, then the forester will rationally choose to reject the bank's offer P_1 and makes her/his own offer. The forester's offer will be close to 200, and the bank will either accept or reject it. According to the game rules, even if the bank rejects the forester's offer, it will no longer have the opportunity to make a new offer.

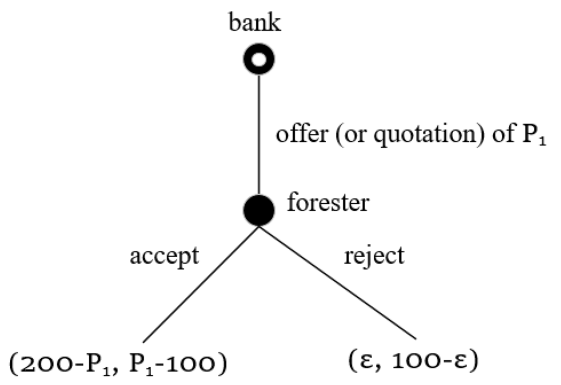

FIGURE 3
Solving for the game equilibrium using backward induction.

The bank could only give up the loan transaction altogether and get zero income. Thence, the forester needs only to offer just under 200 to make the bank's surplus greater than 0.

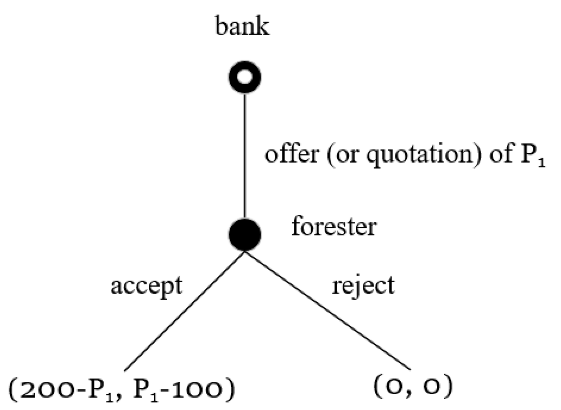

FIGURE 4
A bargaining game in "one-price" mode.

Therefore, in the game shown in Figure 2, when the market powers of the forester and bank are equal to each other, it is obvious that the forester will have more market advantages than the bank does, and the final transaction price of the loan will be close to the reserve price of the bank, which is 200. The bank's surplus is just over zero, and forester's is close to 100.

Banks are more powerful in the mortgage market

In the real world of transactions of forest-title mortgage loans, banks have more advantages over foresters on the market. Because of the limited financing channels available to foresters at the present time, the financing market dominated by loan providers is formed. Meanwhile, forest-resource assets have the characteristics of long growth cycle and high operational risk; they are easily influenced by relevant policies, so that the risk of cashability of forest-resource assets being mortgaged is relatively high. Therefore, in reality, after the bank offers the loan amount that it is willing to grant to the forester, the forester would have no chance to bargain with it one again and can only choose to accept it or give it up altogether. Therefore, the "bargaining game" in the case of equal market power between the two parties in question is transformed into the one in the mode of "one price." The bank holds an absolute advantage in the gameplay and can attain a higher surplus consequently.

In Figure 4, the bank is still the first party to quote. The price offered by the bank is P_1. Because of the disadvantageous position that the forester stands in the mortgage market, the forester has no choice but to either accept the initial price offered by the bank or reject the loan transaction in its entirety. Therefore, the bank's initial offer needs only to make the forester's surplus greater than zero. This scenario can be expressed by using the following equation:

$$P_1 - 100 \succ 0$$

According to the above equation, as long as the bank's initial offer is 100 + ε (where ε is an arbitrarily small positive number), the forester will choose to accept it. At that point, the bank's surplus is 100-ε, and the forester's surplus is ε. In the game shown in Figure 4, the bank is the first and last party to make the offer, so the bank gets a higher surplus, while the forester gets a lower surplus.

To sum up the above points made, when the forester and bank are equally powerful in the mortgage market, the amount of forest-title mortgage loan that the forester is granted by the bank is going to be equivalent to the market value of forest-resource assets that she/he owns, which is equal to 200-ε, and the surplus that she/he gets is 100-ε. When the bank is more powerful in the mortgage market, the forester will only get the loan amount below the market value of forest-resource assets that she/he owns, which is 100 + ε, and she/he will only get a surplus of ε. Therefore, in reality, the loan amount that the forester obtains is going to be lower than the market price of forest-resource assets that he owns. The difference between the market price and the actual loan amount that the forester gets is 100, and the forester's difference in surplus is 100-2ε. The larger the range is for reaching an agreement, the greater the loss of surplus that the forester will undergo.

Game process among forester, bank, and asset-appraisal intermediary

In reality, since the bank holds more advantage than the forester does in the mortgage market, the forester may call upon an asset-appraisal intermediary to assess the value of his forest-resource assets, and then apply for the loan from the bank based on that assessed value. Therefore, the respective game has transformed into the one played by three parties: the forester, the bank, and the asset-appraisal intermediary (Li, 2013).

In this gameplay process, the primary participants of economic behavior switch from two to three parties, namely the forester, the bank, and the asset-appraisal intermediary. The forester would want to use a higher than market value of his assets to obtain a larger amount of the loan, although doing so would greatly increase her/his risk of default of the loan. The bank, on the other hand, would want to multiply this market value by a relatively low discount rate to arrive at the approved loan amount, which is close to the forester's reserve price of the loan. As for the asset-evaluation intermediary, safeguarding the legitimate rights and interests of both other parties is the main purpose of its evaluation of assets. Firstly and most importantly, it must ensure full repayment of the loan granted by the bank to the forester. Secondly, it should see to it that the capital needed to operate the forester's resources be met by the loan. Thirdly, it must also maximize its own business volume, scientifically estimate the value of forest-resource assets owned by the forester, and take the responsibilities that it is supposed to take as an asset-evaluation intermediary.

To continue with the above outcome of the game played by the forester and bank, we assume that $100+\varepsilon$ is the minimum acceptable loan amount equivalent to the value of the forester's forest-resource assets (where ε is an arbitrarily small positive number), and 200-ε is the maximum acceptable loan amount by the bank. Then, the value of assets P which is owned by the forester and evaluated by the asset-appraisal intermediary is:

$$P=(1-\alpha)\times(100+\varepsilon)+\alpha(200-\varepsilon), where 0\prec\alpha\prec 1$$

When constructing the utility functions of the forester, bank, and asset-appraisal intermediary, we need to consider the following three premises: First, the utility of the forester is related to the *p* value; the larger the p value is, the greater the utility of forester is. Therefore, the first derivative of the forester's utility function is greater than zero. When the p value increases to a certain range, the growth rate of utility decreases, hence its second derivative is less than zero. Secondly, the bank's utility is also related to the p value. However, for the bank, the larger the p value is, the smaller the utility of it to the bank. Therefore, the first derivative of the bank's utility function is less than zero. When the p value increases to a certain range, the growth rate of utility decreases, so its second derivative is also less than zero. Finally, as for the asset-appraisal intermediary, its utility is related to that of the forester and bank. The more satisfied both of the other parties are with the transaction results, the higher the utility of asset-appraisal intermediary will be. Therefore, the utility function of the asset-appraisal intermediary is the same as that of the forester and bank. This utility increases with the increase of utilities of the forester and bank, but the marginal cross utility of both parties is negative. Specifically, the utility functions of three primary parties involved in this economic behavior are as follows:

$$\begin{gathered}\text{Forester's utility function:}\\ u=f(P),\ \text{and}\ f'(P)>0, f''(P)\le 0.\end{gathered}$$

$$\text{Bank's utility function}: v=g(P),\ \text{and}\ g'(P)<0, g''(P)\le 0.$$

$$\begin{gathered}\text{Asset-appraisal intermediary's utility function:}\\ s=h(u,v), \text{and}\ \frac{\partial h}{\partial u}>0, \frac{\partial h}{\partial v}>0, \frac{\partial^2 h}{\partial u\partial v}\le 0.\end{gathered}$$

The goal of asset-appraisal intermediary is to maximize its utility. For this reason, we let its first derivative equal to zero and solve for its utility maximization value. The respective calculation can be done as follows:

$$\begin{aligned}&Let: \max h(u,v)\\ &\text{lst derivative}: h'_{\alpha}(u,v)=\frac{\partial h}{\partial u}.\frac{\partial u}{\partial P}.\frac{\partial P}{\partial \alpha}+\frac{\partial h}{\partial v}.\frac{\partial v}{\partial P}.\frac{\partial P}{\partial \alpha}=0\\ &When \frac{\partial h}{\partial u}.\frac{\partial u}{\partial P}=-\frac{\partial h}{\partial v}.\frac{\partial v}{\partial P}, \alpha \text{ is the optimal solution.}\end{aligned}$$

The closer is value of "α" to 1, the closer the assessed value of forest resources is to the maximum acceptable loan amount by the bank, while the forester's surplus is also larger.

The closer is the value of "α" to 0, the closer the assessed value of forest resources is to the minimum acceptable loan amount by the forester, while the bank's surplus is also larger.

As shown in Figure 5 above, (u, v) is the maximum value of utility. When the utility is (u_1, v_1), it means that the bank believes that the asset-appraisal intermediary has overvalued the forest-resource assets that the forester owns, so it would be unwilling to provide the loan in question to the forester, while the forester would be willing to accept it. When the utility is (u_2, v_2), it means that the forester believes that the asset-appraisal intermediary has undervalued the forest-resource assets that she/he owns, so she/he would be unwilling to accept the loan in question from the bank, while the bank would be willing to issue it to the forester. When the utility is (u_3, v_3), it indicates that the value assessed by the intermediary falls into no common interval with that of the forester and bank. That is to say, there is no room for reaching any agreement between the forester and the bank.

As shown in Table 1, (u, v) is the optimal situation of the loan transaction in question, which, in theory, achieves Pareto optimization. However, in reality, we must also take into account the power level of primary parties involved in the economic behavior in the mortgage market. The party with a stronger market power can obtain a more favorable transaction price. Among the current actual transactions of mortgage loans, banks are generally in a stronger position for this. As such, $g'(p)$ would usually move to the left, forming an actual Nash equilibrium of

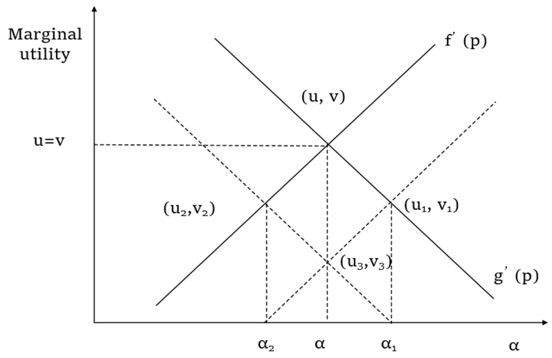

FIGURE 5
A schematic diagram of assessed values.

TABLE 1 Result of game played by the three parties.

Forester bank	To loan	Not to loan
With mortgage	(u,v)	(u_1,v_1)
Without mortgage	(u_2,v_2)	(u_3,v_3)

(u_2, v_2). To summarize the above points, the fairly appraised forest-resource asset value obtained by the forester, bank, and asset-appraisal intermediary through game-playing is called the mortgage value, which is lower than its theoretically Pareto-optimal market value. This value is situated at a low point as its market value changes continuously in the future in reality. It is a conservative and cautious value assessed by the bank which is in a stronger position in the respective mortgage market. While this value is assessed by taking into account the prosperously sustainable operation and cashability of forest-resource assets, it commonly leads to an overly low amount of the loan granted by the bank to the forester in the market, and hence can seriously affect the enthusiasm of foresters applying for this loan. At the same time, it has caused a lack of rural-forestry developmental funds to a certain extent.

Countermeasures and suggestions

Based on the above research findings, we can know the forester is in the lower level than the bank. If the forester would tend to gain mortgage, they need some assistance from social. We intend to make the following suggestions regarding how to increase the limit of forest-title mortgage loan for the forester:

First of all, the government should introduce relevant policies to support the issuance of forest-title mortgage loan. It needs to include this loan into the business category of each of the large commercial banks, and coordinate with the People's Bank of China, China Banking Regulatory Commission, and Insurance Regulatory Bureau in making this type of loan as an area of assessment to the banks. It should also provide the necessary incentive to the banks which have previously launched this type of loan business before. At the same time, it should allow legal and qualified third-party lending institutions to open up such business scopes. In addition, the government should standardize and guide the promotion and development of insurances towards forests through setting up the relevant system. Specifically, this system should consist of the new afforestation, tree seedlings, characteristic economic forests, and under-forest economy, all of which should be included into the subsidy scope of policy-based forest insurance as soon as possible. Furthermore, it' should better continuously improve the policy-based risk compensation mechanism to avoid adverse selection and moral hazard which might occur in the process of issuance of forest-title mortgage loan. With respect to the government's financial policy support, it should further standardize and implement policies regarding discounted interest rates to forestry loans, and constantly increase the depth and breadth of support from central and local governments towards this discounting. Some recent studies show the government should play its main role in the credit risk control of Forest-title mortgage loans, improve the relevant legal system, regulate the behavior of borrowers and lenders, and reduce the profitability and liquidity risks in forest tenure mortgage loans through government encouragement and support. More than that, the government should also improve the compensation and accountability mechanism for the credit risk of forest right mortgage loans, encourage the enthusiasm of forest right mortgage loans through government subsidies, forest insurance and other policies, and ease the problem of forestry financing. According to National Forestry and Grassland Administration, China has issued more than 100 million Forest-title certificates currently. Chinese government is promoting the "separation of ownership, contracting right and management right" of collective forest land, standardizing the transfer of forest rights, innovating the financing mode of forest rights, and improving forest insurance policies.

Secondly, the bank needs to continuously carry on the innovations of credit to be granted to the foresters. In terms of banking products, the bank should enhance its innovation of characteristic forestry loans, and explore the 5-in-1 loan model, which comprises governmental coordination, encouraging enterprises to borrow, encouraging cooperatives to guarantee the loan, encouraging banks disburse the loan, and encouraging foresters to participate in borrowing, so as to reduce the risks that foresters face as they seek finances for their business through taking the loan of forest-title mortgage from the bank. In terms of management, the bank should actively cooperate with village-level cooperative organizations to reduce the cost of bank investigation and improve the efficiency of bank management. In areas with abundant forest resources, the bank could extend its authority of forestry-loan examination and approval to its branches, so as to improve its efficiency of service for issuing forestry credit effectively. Until September 2022, China Development Bank and Agricultural Development Bank granted more than 400 billion yuan of credit, and more than 160 billion yuan of loans to national reserve forest and other forestry loan projects. Forest-title mortgage loans totaled more than 600 billion yuan, and the loan balance was nearly 90 billion yuan.

Finally, foresters can rely on the village-level cooperative organizations to integrate their forest-resource assets and launch scaled operation of them through cooperatives. With an ongoing penetration of China's collective forest-title reform, foresters who independently manage their family-based business gradually realize that this mode of management displays the characteristics of insufficient funds, low profit level, poor risk-prevention ability, and so on. Only through scaled management of their forest-resource assets can they realize the optimal allocation of such resources. Therefore, we should give full play to the role that village cooperative organizations can play in forestry's industrial development and financial services, and stimulate the enthusiasm of cooperative members. In the meantime, by having cooperatives take the lead, we could encourage the foresters to make products and services for the leading forestry enterprises so as to integrate forest-resource assets and form an industrial chain of forestry production and operation. Doing so would also give full play to the core role that leading forestry enterprises will be playing in providing technical support, information service, and scientific management to foresters in the up and downstream of industrial chains. Most importantly, we could provide forest-title mortgage loans to foresters by means of having leading enterprises undertake the loan and cooperatives guarantee it.

Conclusion

In conclusion, this paper first analyzes the game played by the forester and the bank. Then it draws the conclusion as follows: when the forester and the bank have equal power in the market of mortgage loan, the forester will obtain the loan amount equal to the market value of her/his forest-resource assets. When the bank is more powerful in the mortgage market, the forester will only get a loan amount below the market value of his forest-resource assets. After drawing this conclusion, we introduce a new party of asset-appraisal intermediary into our study. Through studying the game played by the forester, the bank, and the asset-appraisal intermediary, we believe that the fair appraisal value of forest-resource assets obtained through gameplay by the 3 parties in question is lower than its original, theoretically Pareto-optimal market value. This lower value is called the mortgage value. It is a conservative and cautious value assessed by the bank which is in a strong position in the forest-title mortgage market. While this value is assessed by taking into account the prosperously sustainable operation and cashability of forest-resource assets, it commonly leads to an overly low amount of the loan granted by the bank to the forester in the market, and hence can seriously affect the enthusiasm of foresters applying for this loan. At the same time, it has caused a lack of rural-forestry developmental funds to a certain extent. Based on the above research findings, we proposes some suggestions regarding how to raise the limit of this loan for the foresters, including elaborating on relevant governmental policies, innovation of credit-related products produced by the banks and relying on the village-level cooperative organizations to integrate forest-resource assets.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. The patients/participants provided their online informed consent to participate in this study, which stated the voluntary nature of participation, and assurance of confidentiality and anonymity.

Author contributions

LX: conceptualization, methodology, data curation, and analysis. WS: writing—original draft preparation, validation, and formal analysis. XL: writing—review and editing. NS: supervision. XZ: investigation and funding acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Deficit irrigation scheduling with mulching and yield prediction of guava (*Psidium guajava* L.) in a subtropical humid region

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Drip irrigation and mulching are often used to alleviate the problem of poor water management in many crops; however, these technologies have not yet been tested for applying water at critical stages of guava orchard growth in subtropical humid Tarai regions of India to improve the yield and quality. A field experiment was conducted over 2020 and 2021 which included three irrigation strategies: severe deficit irrigation (DI_{50}), moderate deficit irrigation (DI_{75}), and full irrigation (FI_{100}), as well as four mulching methods: silver-black mulch (M_{SB}), black mulch (M_B), organic mulch (M_{OM}), and a control without mulch (M_{WM}). The results showed that both the relative leaf water content (RLWC) and the proline content exhibited an increasing trend with a decrease in the irrigation regime, resulting in a 123% increase in the proline content under DI_{50} conditions compared with FI_{100}, while greater plant growth was recorded in fully irrigated plants and using silver-black mulch. Leaf nutrient analysis showed that FI_{100} and M_{OM} produced significantly higher concentrations of all nutrients. However, moderate deficit irrigation (DI_{75}) along with silver-black mulch (M_{SB}) produced higher numbers of fruits per plant, higher average fruit weights, higher fruit yields, and maximum ascorbic acid contents. The irrigation water productivity (IWP) decreased with an increase in the irrigation regime; from severe water deficit to full irrigation, resulting in a 33.79% improvement in IWP under DI_{50} conditions as compared with FI_{100}. Regression analysis outperforms principal component regression analysis for fruit yield prediction, with adjusted R^2 = 89.80%, RMSE = 1.91, MAE = 1.52, and MAPE = 3.83. The most important traits affecting the fruit yield of guava, based on stepwise regression, were leaf proline, leaf Cu, fruit weight, and IWP.

KEYWORDS

guava, deficit irrigation, mulching, plant water relations, irrigation water productivity

1 Introduction

Guava (*Psidium guajava* L.) is one of the most important fruit crops and is widely cultivated in tropical and sub-tropical regions of the world. Guava is known as the "Apple of the tropics" because it is the only tropical fruit which is as nutritionally beneficial as the apple (Khan et al., 2013; Nimisha et al., 2013; Takeda et al., 2022). Over the past two decades, guava land areas and their production have increased at a tremendous pace as the demand for fruits has increased due to their nutritional superiority and affordable prices (Preet et al., 2021). Guava land areas, production, and productivity have increased from 1.55 Mha, 17.15 MT, and 11.10 t/ha, respectively, in 2001 to 2.92 Mha, 43.61 MT, and 14.93 t/ha in 2019–20 (Indiastat-focused on facts). This indicates the mounting importance of this fruit crop as the land area has just less than doubled, with 2.5 times higher production (Anonymous, 2019). In the Uttarakhand plains, its land area has also increased, corresponding to a large area of India; however, the productivity (5.67 t/ha) is still very low as compared with the national average (Department of horticulture and food processing, 2018). The main factor behind the low productivity is poor orchard management practices, resulting in biotic and abiotic stresses (Joshi et al., 2012). Water stress during the critical stages of fruit growth and development is the main reason for poor productivity of guava (Usman et al., 2022). Water management, especially during the period of fruit maturation, plays an important role in improving the yield as well as the quality.

Water shortage is a major barrier in crop production in almost every region of the world (Shao et al., 2009; Bartlett et al., 2019; Kogan et al., 2019). The scarcity of fresh water resources has stimulated research into water-saving strategies in agriculture, with the aim to produce more crop per drop (Stefanelli et al., 2010). India accounts for approximately 18% of the world's population and contains 4% of the world's fresh water, out of which 80% is used in agriculture. According to international criteria, a country is categorized as water-stressed and water-scarce if the per capita water availability falls below 1700 m^3 and 1000 m^3 respectively. India is already a water-stressed country, with 1544 m^3 per capita water availability and is approaching the water-scarce category (Dhawan, 2017). Thus, the main challenge confronting both rain-fed and irrigated agriculture is to improve water use efficiency (WUE) and sustainable water use for agriculture (Berihun, 2011). The use of micro-irrigation systems was found to result in 30–70% water savings in various orchard crops, along with 10–60% increases in yields as compared with conventional methods of irrigation. It is prudent to make efficient use of water and to irrigate larger land areas using the available water resources. This can be achieved by introducing advanced methods of irrigation and improved water management practices (Zaman et al., 2001). In recent years, the implementation of deficit irrigation in various fruit crops has gained popularity due to its excellent influence on water savings, productivity, and produce quality (Galindo et al., 2018).

In order to maximize economic returns while using limited water, deficit irrigation (DI) is used and is the practice of providing irrigation below crop evapotranspiration (ET_c) requirements (Fereres and Soriano, 2007). During various growth stages of the crop, reducing water delivery to the right requirements improves the water use efficiency and produce quality without significantly impacting the yield (Panigrahi et al., 2014). Certain studies have shown that crops can adapt to water shortages, and that a modest water shortage may not have a substantial impact on agricultural productivity (García-Tejero et al., 2010b; Zhong et al., 2019). Scholars have conducted extensive studies to assess the response of fruit tree development to deficit irrigation, most notably on apples (Zhong et al., 2019), almonds (López-López et al., 2018), oranges (Zapata-Sierra and Manzano-Agugliaro, 2017), grapes (Faci et al., 2014), and pear-jujubes (Cui et al., 2008). El Jaouhari et al. (2018) discovered that a moderate deficit irrigation (75% ET_c) can increase the WUE and the quality parameters without sacrificing the yield; however, a severe deficit (50% ET_c) was insufficient to maintain an acceptable fruit size.

Drip irrigation in combination with mulch is one of the best management practices to significantly improve the WUE. Mulching creates an isolating layer between the soil surface and the atmosphere, reducing water vapor interaction between the soil surface and the atmosphere (Zribi et al., 2015). Consequently, water evaporation from mulched soil is reduced compared with bare soil, resulting in more available water for beneficial crop transpiration (Sarkar and Singh, 2007; Hou et al., 2010). Surface mulches have been used to reduce the soil temperature and the wind velocity at the soil surface (Kay, 1978; Jalota and Prihar, 1990).

From a commercial standpoint, VNR Bihi is the dominant guava variety in India, since its larger-sized fruits fetch a decent price in both domestic and foreign markets. This variety's cultivation is mostly limited to central and northern India, where 90% of the annual rainfall occurs in three to 4 months (June–September). Irrigation is mostly used during the fruiting season (September to January) to boost the water productivity and to increase the yield of larger fruits. VNR Bihi guava is, however, well-suited to drip irrigation. One of the key factors for sustaining guava production in this location is to schedule deficit watering with mulching.

Deficit irrigation and mulching have been examined in various field tests as water conservation and water-saving strategies. Furthermore, according to the current literature, there is no information comparing the influence of deficit irrigation with mulching on leaf physiological parameters, nutrient uptake, yield, fruit quality, and irrigation water productivity in guava. Therefore, this study was conducted to determine the best irrigation schedule for VNR Bihi guava, in terms of yield, leaf nutrient content, and irrigation water productivity.

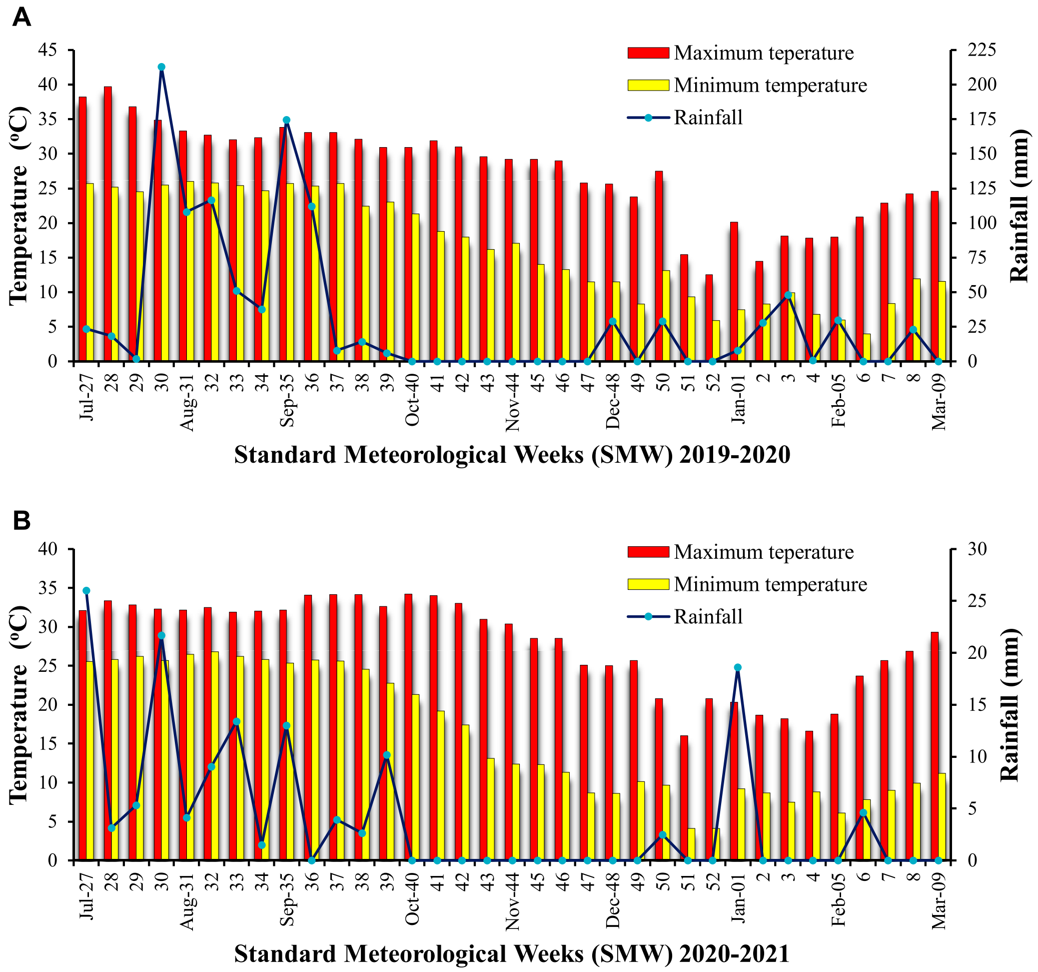

FIGURE 1
Weekly meteorological data during crop growth period for **(A)** 2019–2020 and **(B)** 2020–2021.

2 Materials and methods

2.1 Experimental site

The field experiment was performed at the G. B. Pant University of Agriculture and Technology's Horticulture Research Centre in Uttarakhand, India. The experimental site is located in the Himalayas (29.0 N, 79.5 E). The experiment was conducted on five-year-old guava trees cv. VNR Bihi (wedge grafted) planted at a spacing of 5 m × 3 m under a medium-to high-density planting scheme for two consecutive years (2019–20 and 2020–21). From the first year of planting, the trees were drip irrigated.

The climate of the experimental site has been categorized as sub-humid and sub-tropical with a hot and dry summer and an extremely cold winter. The details of the weather parameters recorded during the crop growth period are depicted in Figures 1A,B. The mean annual rainfall in this region is 1450 mm, out of which 70% occurs during the rainy season (July–September). The total rainfall was 1296.6 and 1252.8 mm during the years 2019–20 and 2020–21, respectively. The soil of the experimental site has been classified as Mollisol. The texture of the experimental soil was silt loam, with a neutral pH (7.1) and EC (0.38 ds/m), medium organic carbon content (0.67%), low available nitrogen (185.95 kg ha^{-1}), and both medium available phosphorus (28.92 kg ha^{-1}) and potassium (220.34 kg ha^{-1}).

2.2 Treatments and layout

The experiment was carried out under natural field conditions in a Factorial Randomized Block Design (FRBD), with three replications comprised of two factors (deficit irrigation and mulching). A total of three irrigation regimes were designed, including severe deficit irrigation

(DI_{50}), moderate deficit irrigation (DI_{75}), and full irrigation (FI_{100}). The irrigation levels were applied on the basis of the crop-evapotranspiration requirement (ET_c): DI_{50}—deficit irrigation at 50% ET_c, DI_{75}—deficit irrigation at 75% Et_c, and FI_{100}—full irrigation at 100% ET_c. Irrigation was applied mainly during the fruit growth period using a drip system. The water supply was stopped during the monsoon season (July–September) due to adequate rainfall fulfilling the crop water need during this period. Four mulching treatments were employed: silver-black mulch (M_{SB}), black mulch (M_B), organic mulch (M_{OM}), and a control without mulch (M_{WM}). Silver-black and black colored polyethylene mulches 100 microns thick and 1.2 m width were used as inorganic mulches (Figure 2A). A 10 cm thick organic mulch (rice straw) was applied uniformly in each replication in the experiment (Figure 2B). Various mulching treatments were given the same quantity of irrigation under the same water deficit schemes.

2.3 Irrigation scheduling and crop management practices

Every other day, four on-line 6 l h^{-1} pressure-compensated drip emitters per plant were installed on two 16 mm diameter lateral pipes to provide irrigation. The emitters were set at a distance of 1 m from the plant stem. Based on a 100% class-A pan evaporation rate, the water quantity applied during full irrigation (FI, 100% ET_c) was estimated using the following formula:

$$ET_c = K_p \times K_c \times E_p \tag{1}$$

where ET_c is the crop evapotranspiration (mm/day), K_p is the pan coefficient (0.7), E_p is the 2-day cumulative pan evaporation (mm), and K_c is the crop coefficient (0.8 for no mulching and 0.56 for mulches). The K_c values decrease by an average of 10–30% due to the 50–80% reduction in soil evaporation under mulching (Allen et al., 1998). The volume of water applied by the drip irrigation system was estimated using the following relationship:

FIGURE 2
Guava plants with **(A)** polyethylene mulches and **(B)** organic mulch treatments.

TABLE 1 The crop coefficient (Kc), rainfall and irrigation applied during irrigation season under different irrigation and mulching treatments.

Treatments	K_c	E_{pan}	Rainfall (mm)	Effective rainfall (mm)	Irrigation water applied per plant (mm)
2019–2020					
FI_{100} + Mulch	0.56	455.2	541	338.53	802.94
FI_{100} + Without mulch	0.8	455.2	541	338.53	808.56
DI_{75} + Mulch	0.56	455.2	541	338.53	602.21
DI_{75} + Without mulch	0.8	455.2	541	338.53	606.42
DI_{50} + Mulch	0.56	455.2	541	338.53	401.47
DI_{50} + Without mulch	0.8	455.2	541	338.53	404.28
2020–2021					
FI_{100} + Mulch	0.56	552.9	82	76.31	975.31
FI_{100} + Without mulch	0.8	552.9	82	76.31	1316.97
DI_{75} + Mulch	0.56	552.9	82	76.31	731.48
DI_{75} + Without mulch	0.8	552.9	82	76.31	987.73
DI_{50} + Mulch	0.56	552.9	82	76.31	487.66
DI_{50} + Without mulch	0.8	552.9	82	76.31	658.49

K_c, crop coefficient; Epan, pan evaporation; DI_{50}, deficit irrigation at 50% ETc; $DI_{75,}$ deficit irrigation at 75% ET_c; FI_{100} - full irrigation at 100%; ET_c; ET_c, crop evapotranspiration.

$$V = \sum\left(E_p \times K_p \times K_C \times S_p \times S_r \times W_p - E_R\right) \quad (2)$$

where V = the total amount of water applied (L/day/plant), E_p = the open pan evaporation (mm/day), K_p = the pan coefficient, K_c = the crop coefficient, S_p = the plant to plant spacing, S_r = the row to row spacing, W_p = the wetting factor, and E_R = the effective rainfall. The irrigation efficiency of the drip was considered to be 90%. The effective rainfall was calculated monthly, based on the USDA, S.C.S. method (United States Department of Agriculture, Soil Conservation Services):

$$ER = P_t\left[\frac{125 - 0.2 \times P_t}{125}\right] \text{for } P_t < 250\,\text{mm} \quad (3)$$

$$ER = 125 + 0.1 \times P_t \text{ for } P_t < 250\,\text{mm} \quad (4)$$

where ER = the effective rainfall (mm) and P_t = the total rainfall (mm).

The crop coefficient (K_c), rainfall, and irrigation applied during the irrigation season, under different irrigation treatments, are shown in Table 1. One pair leaf pruning was practiced in the last week of April to regulate rainy season flush and to optimize winter season flowering. Standard recommended doses of fertilizer, i.e., $N:P_2O_5:K_2O$ at 375:325:250 g/tree/year were applied during both years.

2.4 Measurement and analysis

2.4.1 Leaf physiological parameters

For determining the relative leaf water content (RLWC), two leaves per plant in a similar position were cut from each shoot at midday. The RLWC was calculated using the formula given by Bowman (1989):

$$\text{RLWC}\,(\%) = \left[\frac{(FW - DW)}{TW - DW}\right] \times 100 \quad (5)$$

The proline content of the guava leaves was estimated according to the procedure of Bates et al. (1973). The total chlorophyll content was estimated in fresh guava leaves using the method described by Hiscox and Israelstam (1979). The total chlorophyll content was then calculated by using following formula:

$$\text{Total chlorophyll} = \left[\frac{(20.2 \times A645) + (8.02 \times A663) \times V}{\text{Weight }(g) \times 1000}\right] \quad (6)$$

where, A = the absorbance of chlorophyll extract at a specific indicated wavelength, V = the final volume of the sample, and W = the fresh weight of tissue extracted.

Towards the end of each irrigation season, fully expanded, mature leaves (without petioles) were collected from the plant canopy for each treatment and analyzed for macronutrients (N, P, and K) and micronutrients (Fe, Mn, Cu, and Zn). Two leaves displaying opposite phyllotaxy and emerging simultaneously were considered as a single leaf. As the majority of the shoots (95%) contained six leaf pairs, leaves from six different positions were sampled and indicated as leaf position I-VI from the base to the top. The leaves were spread in all four directions and were located at a height of 0.5–2 m above the soil level. The sample sizes consisted of 20 leaves per sample per replication. The leaf samples were thoroughly washed and dried at 65°C for 48 h. The dried samples were homogenously powdered and digested in a tri-acid mixture made up of two parts $HClO_4$ + five parts HNO_3 + one part H_2SO_4. Leaf acid extracts were analyzed for N using the modified micro-Kjeldahl method, P using the vanadomolybdo-phosphoric acid method, K using flame photometry, and micronutrients (Fe, Mn, Cu, and Zn) using an atomic absorption spectrophotometer (Model-908, GBC Scientific equipment, Australia).

The plant height, average plant spread (mean diameter of the canopy spread in E–W and N–S directions), and stem girth diameter (stem diameter measured 50 cm above the ground surface) were recorded annually. The plant canopy volume was calculated using the following formulae and was expressed in cubic meters (m^3):

$$\text{Canopy volume} = \tfrac{4}{3}\,[\pi r^2 h] \qquad (7)$$

where, r = the radius of the plant canopy (m), h = the total height of the plant, and π = 3.14.

The numbers and weights of complete fruits collected from each experimental plant were recorded, and the mean yield per plant was computed by multiplying the number of fruits per plant by the average fruit weight undergoing the various treatments (Figure 4). The fruit lengths and fruit diameters were measured using a Digital Vernier's Caliper for ten fruits/replication, randomly selected from tagged guava fruits grown under different treatments. The fruit yield per unit quantity of irrigation water applied was calculated to determine the irrigation water productivity (IWP). The ascorbic acid content of the guava fruits was determined by using a 2,6-dicholorophenol-indophenol visual titration method described by Ranganna (1986). Ten grams of pulp was weighed and crushed using a mortar and pestle. The juice was filtered from the crushed pulp into a 100 ml volumetric flask and the final volume was made up to 100 ml by adding 3% metaphosphoric acid (HPO_3) solution. A 10 ml aliquot was taken from the obtained solution and was used for titration against the dye (2,6-dicholorophenol-indophenol) until a light pink color appeared and persisted for at least for 15 s (end point). The titer value was recorded, and the ascorbic acid content was calculated and expressed as mg/100 g of pulp:

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titre value} \times \text{dye factor} \times \text{volume made up} \times 100}{\text{Weight or volume of sample taken} \times \text{volume of aliquot taken}} \qquad (8)$$

$$\text{Dye factor} = \frac{0.5}{\text{Titre value}} \qquad (9a)$$

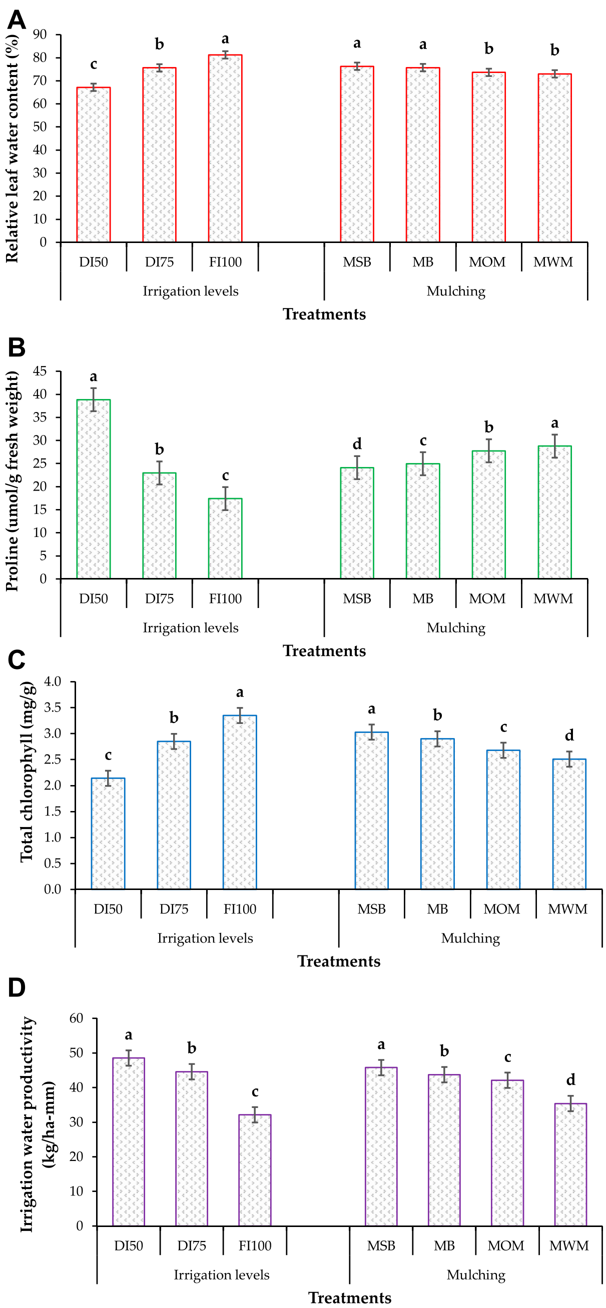

FIGURE 3
(A) Relative leaf water content (RLWC), **(B)** proline content, **(C)** total chlorophyll content and **(D)** irrigation water productivity influenced by irrigation levels and mulching. Different letters **(A–D)** indicate significantly different values at $p \leq 0.05$ according to Duncan's multiple range test. The vertical bar at each data point represents the standard error of mean.

2.5 Statistical analysis

Statistical analysis of two FRBDs was carried out by using the R package (Popat and Banakara, 2020). The means of the levels of significant factors were compared using Duncan's new multiple range test (Tallarida and Murray, 1987). The graphs presented in this manuscript were prepared using the R package ggbiplot2 (Wickham, 2016). The Pearson correlation was determined for all variables in the study (Obilor and Amadi, 2018). The significance of correlation was tested using a t-test (n = 72).

Stepwise regression analysis was performed by using the fruit yield (kg/plant) as the dependent variable, and remaining variables were used as independent variables. Residual analysis of the regression model was performed by using a run test (Siegel, 1988) and a Shapiro–Wilk test (Shapiro and Wilk, 1965) was used to test the assumptions of randomness and the normality of residuals. Goodness of fit statistics such as the RMSE (root mean squared error), MAE (mean absolute error), and MAPE (mean absolute percentage error) were determined for the regression model. All 20 variables were first subjected to principal component regression analysis using the R package prcomp. Among the principal components obtained, those with eigen values greater than 1 were used as independent variables and the yield was used as the dependent variable. All analyses were performed using R software, version 4.0.5 (Shukla et al., 2021; Elbeltagi et al., 2022).

3 Results and discussion

3.1 Leaf physiological parameters

The mean relative leaf water contents (RLWC) under different irrigation treatments were affected significantly (Figure 3A). The highest values for the RLWCs (81.35% and 81.10%) were observed for fully irrigated plants, whereas the lowest values were observed for plants under DI50 (67.75% and 66.58%), in 2019–20 and 2020–21, respectively. However, the lower values of the RLWC in 2020 compared with those in 2019 reflect the higher plant water status in the former year. The lower atmospheric evaporative demand, coupled with the better rainfall distribution in 2019 are probably responsible for the higher plant water potential in 2019.

The increased values of the RLWC indicates that the plant received its required water level to accomplish different plant physiological functions. The RLWC is an integrative index of the plant water status, and is used to evaluate the plant's tolerance to water stress. A reduction in the RLWC under water stress leads to stomatal closure, resulting in decreased CO_2 assimilation (Gindaba et al., 2004). Similar results were also reported by Abdel-Razik (2013) and Khalifa (2013) in mangos, and by Hamdy et al. (2016) in pomegranates; the authors reported that the plants produced low values of relative leaf water content in deficit irrigation conditions.

Different mulches also substantially influenced the RLWC of guava plants. The highest values for the RLWCs were observed with MSB, which was statistically at par with MB, whereas the lowest value was observed for MWM plants during both years of experimentation (Figure 3A). Moreover, interactive effects between deficit irrigation and mulching, with respect to the relative leaf water content, were significant during both years. Pooled data for the 2 years revealed that the highest value for the RLWC (82.31%) was observed with FI100MB (full irrigation at 100% ETc and with black mulch), whereas the lowest value of the RLWC (63.09%) was observed with plants under DI50MWM conditions (deficit irrigation at 50% ETc without mulch). These findings are in accordance with the findings of Pratima (Pratima, 2014), who reported significantly higher relative leaf water content in kiwifruits under deficit irrigation conditions coupled with black polyethylene mulching as compared with un-mulched conditions.

The proline accumulation in leaves significantly increased when plants were irrigated at DI50 in comparison with fully irrigated plants, over both years of the experiment (Figure 3B). During 2019–20, the average leaf proline content increased from 17.29 μmol/g fresh weight under FI100 conditions to 38.80 μmol/g fresh weight when plants were irrigated at DI50 conditions.

Similarly, in 2020–21, the leaf proline content increased from 17.52 μmol/g fresh weight in FI100 conditions to 38.88 μmol/g fresh weight under DI50 conditions. The proline content of leaves increased as the irrigation water decreased, implying that proline production is a common response of plants that are under water stress, as proline regulates cell osmotic equilibrium and protects against the harmful effects of water stress (Sampathkumar et al., 2014). Similar findings were also reported by Srikasetsarakul et al. (2011) in mangos and by Teixeira and Pereira (2007) in potatoes. Moreover, the minimum leaf proline content (24.05 μmol/g fresh weight) was recorded in MSB (silver-black) mulch, whereas the maximum (28.68 μmol/g fresh weight) in MWM (without mulch) plants. Similarly, in 2020–21, the minimum leaf proline content (24.17 μmol/g fresh weight) was recorded in MSB (silver-black) mulch, whereas the maximum (28.86 μmol/g fresh weight) in MWM (without mulch) plants. The interaction effect of deficit irrigation and mulching on the leaf proline contents was non-significant during both years.

Leaf chlorophyll content is a vital component for photosynthesis, and indicates the amount of photosynthates present in the plant system, which help to regulate plant growth. The total chlorophyll content was significantly affected by deficit irrigation and mulching treatments during both years of experimentation (Figure 3C). Pooled data indicated that plants irrigated at 100% ETc exhibited the highest chlorophyll content: 3.35 mg g−1, whereas the minimum (2.14 mg g−1) was recorded for DI50 conditions. The reduction in chlorophyll content with respect to water deficit may be due to the fact that photosynthetic pigments are very sensitive to drought stress, resulting in the destruction of chlorophyll. Under stressful conditions, glutamate, which is a fundamental source for both chlorophyll and proline formation, is thought to be primarily utilized for proline production, as a protectant suitable solute (Jaleel et al., 2009). Furthermore, during shortage irrigation, activation of the chlorophyllase enzyme can produce a drop in chlorophyll concentrations (Farooq et al., 2009).

Plants mulched with silver-black (MSB) registered significantly higher total leaf chlorophyll contents as compared with black mulch and MWM (without mulch) during both years (Figure 3C). The increased total chlorophyll might be due to the fact that the plastic mulching increased the soil microbial population along with the nitrogen absorption, consequently increasing the chlorophyll content of the plant leaves, as mentioned by Eissa (2002). The greater chlorophyll concentration in plants growing on polyethylene mulch could be due to a difference in chlorophyll synthesis and breakdown. Furthermore, the greater activity of the enzyme chlorophyllase could be related to the lower chlorophyll contents in non-mulched leaves. Differences in chlorophyll levels could also be attributable to differences in the degree of light reflection by the mulches (Pandey et al., 2016; Farooq et al., 2021, 2022; Kumar et al., 2022). Similar results were also reported by Deb et al. (2014), who recorded maximum total chlorophyll contents in polyethylene mulches in strawberries.

3.2 Leaf nutrient composition

The macronutrient (N, P, and K) and micronutrient (Cu, Mn, Zn, and Fe) concentrations in leaves responded differently to various irrigation regimes, according to leaf nutrient analyses (Tables 2, 3). The FI100 regime produced significantly higher leaf N, P, and K contents, which were statistically comparable to the DI75 condition, but significantly higher than the DI50 regime. The increased availability of such nutrients in the soil under FI100 resulted in higher N, P, and K contents in leaves of completely irrigated plants. The decrease in leaf nitrogen content under deficit irrigation conditions could be due to a decrease in the nitrogen solubility caused by soil water stress: the plant does not absorb enough nitrogen (Tahir et al., 2003). Similar results were also reported by Khattab et al. (2011); the leaf nitrogen percentage of pomegranates increased with increasing irrigation water levels compared with drought stress conditions, and the available N in the soil (NO_3^- and NH_4^+), nitrogen fixation, uptake, and nitrogen use efficiency were significantly reduced, leading to lower nitrogen accumulation in plants. These findings agree with the earlier studies of Panigrahi et al. (2012) on Kinnow mandarins, Gupta, (2019) on litchi, and Preet et al. (2021) on the response of guava to integrated nutrient and water management. Leaf N, P, and K contents also significantly differed when guava plants were mulched using silver-black, black, and organic mulch (rice straw) for two consecutive years. Furthermore, mulching with different types of mulches significantly influenced the leaf N, P, and K contents; a maximum was observed for MOM, followed by MSB, and a minimum for MWM over two consecutive years. The interaction effects of deficit irrigation and mulching had non-significant influences on the N, P, and K contents of leaves during both years of experimentation.

Moreover, the highest concentrations of micronutrients were registered for FI100 conditions (Cu, 15.64–16.21 ppm; Mn, 60.01–61.13 ppm; Zn, 48.67–48.97 ppm; and Fe, 192.33–193.36 ppm), followed by DI75 conditions (Cu, 13.52–14.07 ppm; Mn, 56.01–56.48 ppm; Zn, 43.59–43.85 ppm; and Fe, 179.70–180.39 ppm), and the minimum were recorded for DI50 conditions (Cu, 11.29–11.77 ppm; Mn, 53.32–53.59 ppm; Zn, 40.91–41.18 ppm; and Fe, 171.90–172.41 ppm), presented in Table 3. The increased availability of micronutrients under full irrigation conditions might be attributed to a low redox potential due to a low oxygen content, the increased solubility of the reduced form of iron (Fe^{3+} to Fe^{2+}), and other micronutrients in soil (Marathe et al., 2009). Increased micronutrient concentrations in leaves under the full irrigation regime were also reported by Khan et al. (2013) for guava, Panigrahi et al. (2014) for Kinnow mandarins, and Nadu (2018) for pomegranates. Various mulches also significantly influenced the leaf micronutrient concentrations (Cu, Mn, Zn, and Fe) during 2019–20 and 2020–21. The highest concentrations of the micronutrients were registered for MWM, followed by MSB, while the minimum concentrations of micronutrients was recorded in un-mulched plants. Barman et al. (2017), for guava cv. Lalit, also reported increased concentrations of micronutrients under mulched conditions.

3.3 Plant vegetative growth

Different growth parameters such as plant height, average plant spread, stem girth diameter, canopy volume, and leaf area were significantly influenced by different irrigation treatments and mulch types (Table 4). Among the irrigation regimes, FI100 treatment registered significantly higher plant heights, average plant spreads, stem girth diameters, canopy volumes, and leaf areas, whereas minimum values were observed for DI50 conditions during both years. The increased ABA biosynthesis in the roots and the reduction of cytokinin synthesis in the roots, branches, and buds in deficit irrigation conditions affects the vegetative growth of plants (Dodd, 2005). Earlier studies by Panigrahi et al. (2014) on Kinnow mandarins also showed similar findings; a decrease in vegetative growth upon deficit irrigation treatment.

The maximum plant height was recorded for MSB, and the minimum under MWM conditions. Moreover, MSB, MB, and MOM were statistically at par with one another during both years of experimentation. On the basis of pooled data, the canopy volume was 27.59% higher for MSB compared with MWM. The leaf area was also significantly higher for MSB: 8.56% higher than that of MWM. The increased plant growth parameters due to mulching might be caused by higher plant physiological processes as congenial moisture and a range of temperatures were available over the experimental period (Khan et al., 2013). Moreover, optimum moisture availability in silver-black

TABLE 2 Macronutrient (N, P and K) content in leaves of VNR Bihi guava influenced by irrigation levels and mulching.

Treatments	Leaf N (%)			Leaf P (%)			Leaf K (%)		
	2019–2020	2020–2021	Pooled	2019–2020	2020–2021	Pooled	2019–2020	2020–2021	Pooled
(A) Irrigation levels									
DI50	1.90[c]	1.95[c]	1.92[c]	0.174[c]	0.175[c]	0.175[c]	0.95[c]	0.96[c]	0.95[c]
DI75	1.97[b]	2.03[b]	2.00[b]	0.212[b]	0.216[b]	0.214[b]	1.02[b]	1.04[b]	1.03[b]
FI100	2.06[a]	2.14[a]	2.10[a]	0.231[a]	0.235[a]	0.233[a]	1.16[a]	1.18[a]	1.17[a]
(B) Mulching									
Silver-black	2.00[ab]	2.05[ab]	2.02[ab]	0.207[ab]	0.211[ab]	0.209[b]	1.05[ab]	1.07[ab]	1.06[b]
Black	1.96[ab]	2.03[ab]	1.99[bc]	0.203[b]	0.206[b]	0.205[b]	1.04[ab]	1.06[b]	1.05[b]
Organic	2.03[a]	2.10[a]	2.06[a]	0.215[a]	0.219[a]	0.217[a]	1.08[a]	1.10[a]	1.09[a]
Without mulch	1.93[b]	1.98[b]	1.95[c]	0.198[b]	0.197[c]	0.198[c]	1.01[b]	1.02[c]	1.01[c]
Interaction (A × B)	NS	NS	NS	NS	NS	NS	NS	NS	NS

N, nitrogen; P, phosphorus; K, potassium; DI_{50}, deficit irrigation at 50% ETc; DI_{75}, deficit irrigation at 75% ETc; FI_{100}, full irrigation at 100% ETc. Values marked by a different letter differ significantly according to Duncan's multiple range test ($p \leq 0.05$).

mulch maintains proper turgor pressure, required for stomatal opening for gaseous exchange, which eventually led to a higher photosynthetic rate (Ayotamuno et al., 2007). The above findings are in agreement with the results of Singh et al. (2020) and Preet et al. (2021); the authors reported maximum canopy volumes with silver-black mulch as compared with no mulch in guava cv. VNR Bihi.

3.4 Yield parameters

Table 5 presents the numbers of fruits per plant, average fruit weights, fruit yields, fruit lengths, and fruit diameters produced under various irrigation regimes and mulches. The number of fruits harvested per plant and the mean fruit weights increased with increasing irrigation regime, i.e., from 50% ETc to 75% ETc under DI conditions, and were slightly lower in FI100 (full irrigation at 100% ETc) as compared with DI75 (deficit irrigation at 75% ETc) during both years. However, regarding the fruit weights, FI100 and DI75 treatments were statistically at par with each other during both years of the study. Moreover, the number of fruits harvested per plant was higher in 2019 than in 2020, due to better flowering, higher average rainfall, and lower fruit cracking in 2019 than in 2020. In contrast, the mean fruit weights were higher in 2020 than in 2019, due to a lower number of fruits per plant in 2020 than in 2019. Intrigliolo et al. (2013) reported a significant increase in the fruit numbers and fruit weights for pomegranates under deficit irrigation conditions; the authors concluded that mitigated competition between vegetative growth and reproductive organs, caused by mild water stress, reduced the abscission of reproductive organs. Drip irrigation provides a consistent soil moisture regime in which roots remain active throughout the season, resulting in an optimum availability of nutrients and proper translocation of food materials, which accelerates fruit growth and development in guava. The authors recorded a maximum fruit weight at 80% irrigation using plastic mulching in guava cv. Allahabad Safeda (Singh et al., 2015). Panigrahi et al. (2012) also reported similar decreased mean fruit weights as the irrigation regime decreased from 80% Ecp to 40% Ecp under DI conditions, in Nagpur mandarins.

The highest fruit yields were recorded for DI75 (44.51 and 42.93 kg/plant), followed by FI100 (46.64 and 44.69 kg/plant) during 2019–20 and 2020–21, respectively. The fruit yields under different irrigation treatments were higher in 2020 than in 2019, due to higher fruit weights in the former year. On the basis of pooled data, the fruit yields increased by almost 37.54% upon increasing the irrigation level from 50% ETc to 75% ETc under DI. The possible reasons for higher fruit yields under DI75 might be that the water deficit (20–25% available soil water depletion) in the root zone under this treatment suppressed vegetative growth of the plants without much effect on the leaf photosynthesis rate; plants invested higher quantities of photosynthates towards reproductive growth (fruiting) than vegetative growth (Panigrahi et al., 2012). As guava is a hardy plant and can be grown in semi-arid and arid zones under water stress conditions, this might be a reason why plants irrigated with moderate water deficits (deficit irrigation at 75% ETc) performed better as compared with full irrigation. Similar results were reported by Kaushik et al. (2013), Singh et al. (2015), and Preet et al. (2021) in guava.

Mulching significantly influenced the guava plant yields during both years of study. MSB exhibited almost 14.19% and 16.46% higher fruit yields during 2019–20 and 2020–21, respectively, as compared with un-mulched plants (Table 5). The positive impact on yield parameters due to various mulches might be attributed to an alteration of the microclimate in favor of the guava plants *viz.*, temperature regulation, maintenance of appropriate soil moisture status, as well as reduced weed

TABLE 3 Micronutrients (Cu, Mn, Zn and Fe) content in leaves of VNR Bihi guava influenced by irrigation levels and mulching.

Treatments	Leaf Cu (ppm)			Leaf Mn (ppm)			Leaf Zn (ppm)			Leaf Fe (ppm)		
	2019–2020	2020–2021	Pooled	2019–2020	2020–2021	Pooled	2019–2020	2020–2021	Pooled	2019–2020	2020–2021	Pooled
(A) Irrigation levels												
DI50	11.29^{c}	11.77^{c}	11.53^{c}	53.32^{c}	53.59^{c}	53.45^{c}	40.91^{c}	41.18^{c}	41.05^{c}	171.90^{c}	172.41^{c}	172.16^{c}
DI75	13.52^{b}	14.07^{b}	13.79^{b}	56.01^{b}	56.48^{b}	56.24^{b}	43.59^{b}	43.85^{b}	43.72^{b}	179.70^{b}	180.39^{b}	180.04^{b}
FI100	15.64^{a}	16.21^{a}	15.92^{a}	60.01^{a}	61.13^{a}	60.57^{a}	48.67^{a}	48.97^{a}	48.82^{a}	192.33^{a}	193.36^{a}	192.85^{a}
(B) Mulching												
Silver-black	13.79^{b}	14.39^{b}	14.09^{b}	56.84^{ab}	57.77^{a}	57.31^{ab}	45.10^{ab}	45.32^{ab}	45.21^{ab}	182.66^{ab}	183.89^{ab}	183.28^{ab}
Black	13.30^{c}	13.90^{b}	13.60^{c}	56.02^{ab}	56.72^{ab}	56.37^{bc}	43.91^{bc}	44.28^{b}	44.10^{b}	179.78^{ab}	180.47^{ab}	180.13^{bc}
Organic	14.47^{a}	14.93^{a}	14.70^{a}	58.48^{a}	59.01^{a}	58.75^{a}	46.30^{a}	46.60^{a}	46.45^{a}	185.77^{a}	186.52^{a}	186.14^{a}
Without mulch	12.37^{d}	12.84^{c}	12.61^{d}	54.43^{b}	54.76^{b}	54.60^{c}	42.23^{c}	42.47^{c}	42.35^{c}	177.02^{b}	177.34^{b}	177.18^{c}
Interaction (A × B)	S	S	S	NS	NS	NS	NS	NS	NS	NS	NS	NS

Cu, copper; Mn, manganese; Zn, zinc; Fe, iron; DI_{50}, deficit irrigation at 50% ETc; $DI_{75,}$ deficit irrigation at 75% ETc; FI_{100} - full irrigation at 100% ETc. Values marked by a different letter differ significantly according to Duncan's multiple range test ($p \leq 0.05$).

TABLE 4 Plant growth parameters of VNR Bihi guava influenced by irrigation levels and mulching.

Treatments	Plant height (m)			Canopy spread (m)			Canopy volume (m^{3})			Stem girth (cm)			Leaf area (cm^{2})		
	2019–2020	2020–2021	Pooled	2019–2020	2020–2021	Pooled	2019–2020	2020–2021	Pooled	2019–2020	2020–2021	Pooled	2019–2020	2020–2021	Pooled
(A) Irrigation levels															
DI50	3.76^{c}	3.88^{c}	3.82^{c}	2.77^{c}	3.39^{c}	3.08^{c}	30.51^{c}	46.77^{c}	38.64^{c}	9.56^{c}	10.46^{c}	10.01^{c}	44.38^{c}	42.83^{c}	43.61^{c}
DI75	3.93^{b}	4.07^{b}	4.00^{b}	3.22^{b}	3.81^{b}	3.52^{b}	42.86^{b}	61.98^{b}	52.42^{b}	10.62^{b}	11.79^{b}	11.20^{b}	48.60^{b}	49.55^{b}	49.08^{b}
FI100	4.06^{a}	4.21^{a}	4.14^{a}	3.49^{a}	4.08^{a}	3.78^{a}	52.04^{a}	73.48^{a}	62.76^{a}	11.50^{a}	13.13^{a}	12.32^{a}	53.40$_{a}$	54.81^{a}	54.10^{a}
(B) Mulching															
Silver-black	4.02^{a}	4.14^{a}	4.08^{a}	3.35^{a}	3.94^{a}	3.64^{a}	47.67^{a}	67.83^{a}	57.75^{a}	11.14^{a}	12.46^{a}	11.80^{a}	50.76^{a}	51.15^{a}	50.95^{a}
Black	3.93^{ab}	4.07^{ab}	4.00^{ab}	3.21^{b}	3.80^{ab}	3.50^{b}	42.91^{b}	62.37^{b}	52.64^{b}	10.98^{a}	12.16^{a}	11.57^{a}	49.59^{ab}	49.99^{ab}	49.79^{ab}
Organic	3.89^{ab}	4.03^{ab}	3.96^{bc}	3.11^{bc}	3.71^{bc}	3.41^{b}	40.07^{c}	58.80^{c}	49.44^{c}	10.31^{ab}	11.59^{ab}	10.95^{b}	47.91^{b}	48.19^{bc}	48.05^{bc}
Without mulch	3.83^{b}	3.96^{b}	3.90^{c}	2.99^{c}	3.58^{c}	3.29^{c}	36.55^{d}	53.96^{d}	45.26^{d}	9.81^{b}	10.96^{b}	10.38^{b}	46.92^{b}	46.94^{c}	46.93^{c}
Interaction (A × B)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

DI_{50}, deficit irrigation at 50% ETc; $DI_{75,}$ deficit irrigation at 75% ETc; FI_{100}, full irrigation at 100% ETc. Values marked by a different letter differ significantly according to Duncan's multiple range test ($p \leq 0.05$).

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TABLE 5 Number of fruits harvested, average fruit weight, fruit yield, fruit length and fruit diameter of VNR Bihi guava influenced by irrigation levels and mulching.

Treatments	No. of fruits/plant			Fruit weight (g)			Fruit yield (kg/plant)			Fruit length (cm)			Fruit diameter (cm)		
	2019–2020	2020–2021	Pooled	2019–2020	2020–2021	Pooled	2019–2020	2020–2021	Pooled	2019–2020	2020–2021	Pooled	2019–2020	2020–2021	Pooled
(A) Irrigation levels															
DI_{50}	73.50^{b}	64.75^{c}	69.13^{c}	437.80^{b}	526.19^{b}	482.00^{b}	32.18^{c}	34.08^{c}	33.13^{c}	7.84^{c}	8.71^{c}	8.28^{c}	8.49^{c}	9.22^{c}	8.85^{c}
DI_{75}	88.00^{a}	77.50^{a}	82.75^{a}	504.96^{a}	601.02^{a}	552.99^{a}	44.51^{a}	46.64^{a}	45.57^{a}	8.80^{a}	9.73^{a}	9.27^{a}	9.40^{a}	9.98^{a}	9.69^{a}
FI_{100}	85.75^{a}	75.00^{b}	80.38^{b}	500.54^{a}	595.67^{a}	548.10^{a}	42.93^{b}	44.69^{b}	43.81^{b}	8.42^{b}	9.30^{b}	8.86^{b}	9.08^{b}	9.65^{b}	9.36^{b}
(B) Mulching															
Silver-black	85.33^{a}	74.67^{a}	80.00^{a}	495.24^{a}	592.98^{a}	544.11^{a}	42.55^{a}	44.57^{a}	43.56^{a}	8.62^{a}	9.42^{a}	9.02^{a}	9.17^{a}	9.78^{a}	9.47^{a}
Black	83.33^{ab}	73.33^{a}	78.33^{ab}	486.03^{ab}	576.08^{ab}	531.06^{ab}	40.75^{b}	42.44^{b}	41.59^{b}	8.44^{ab}	9.31^{ab}	8.88^{ab}	9.05^{ab}	9.67^{ab}	9.36^{ab}
Organic	81.33^{ab}	71.67^{ab}	76.50^{bc}	476.77^{bc}	568.94^{b}	522.86^{bc}	38.94^{c}	40.91^{b}	39.92^{c}	8.26^{ab}	9.19^{ab}	8.73^{bc}	8.94^{ab}	9.55^{b}	9.25^{bc}
Without mulch	79.67^{b}	70.00^{b}	74.83^{c}	466.36^{c}	559.16^{b}	512.76^{c}	37.26^{d}	39.29^{c}	38.27^{d}	8.09^{b}	9.06^{b}	8.57^{c}	8.80^{b}	9.47^{b}	9.13^{c}
Interaction (A × B)	NS	NS	NS	NS	NS	NS	S	S	S	NS	NS	NS	NS	NS	NS

DI_{50}, deficit irrigation at 50% ETc; DI_{75}, deficit irrigation at 75% ETc; FI_{100}, full irrigation at 100% ETc. Values marked by a different letter differ significantly according to Duncan's multiple range test ($p \leq 0.05$).

competition, soil compaction, and erosion, generating enhanced moisture and nutrient availability in the fruit plants. These favorable factors undoubtedly improved the guava plant yields (Singh et al., 2020). The interactive relationship between deficit irrigation and mulch, i.e., DIxM, and its effect on the fruit yield/plant varied and were statistically significant in both consecutive years (Table 6). On the basis of pooled data for the 2 years, it is pertinent to mention that the fruit yield/plant improved by 36.81% in guava plants irrigated under DI75MSB as compared with DI50MWM. Singh et al. (2015) also revealed higher fruit yields in guava cv. Allahabad Safeda., treated with drip irrigation at 80% Ecp and using plastic mulching.

The average fruit lengths and diameters were significantly lower in 2019–20 compared with 2020–21. In both years, these parameters were highest for DI75 treatment and were significantly different from the FI100 and the DI50 treatments. It is also clear from the pooled data for two consecutive years that fruit lengths and diameters improved by 10.67% and 8.66%, respectively, and were improved under DI75 as compared with DI50 conditions. The increased fruit lengths and diameters under moderate water deficit conditions might be due to balanced vegetative growth and maximum interception of light, as elucidated by Kumawat et al. (2017) for guava. Similar findings for guava were also reported by Kaushik et al. (2013) and Preet et al. (2021). Likewise, different types of plant mulches also significantly influenced the fruit lengths and widths during both years of investigation. Pooled data indicated that plants mulched with MSB exhibited substantially higher fruit lengths (9.02 cm) and fruit diameters (9.47 cm), followed by MB, and minimum values observed for un-mulched plants. The positive impact of various mulches on the fruit length might be due to the fact that the mulches provided consistently improved available soil moisture in the plant basin, in which the plant roots remained active throughout the season, resulting in optimum availability of nutrients and proper translocation of food materials, which accelerated fruit growth and development (Joshi et al., 2011). Higher fruit lengths and diameters caused by silver-black mulch were also reported by Singh (2020) in VNR Bihi guava and by Beelagi (2020) in pomegranates.

3.5 Irrigation water productivity

The irrigation water productivity was significantly affected by various deficit irrigation levels during both years of experimentation (Figure 3D). The irrigation water productivity decreased as the irrigation regime increased from DI50 to FI100. The irrigation water productivity improved by 33.27% and 34.43% under DI50 as compared with FI100 during 2019–20 and 2020–21, respectively. The rate of water loss through evaporation from the soil surface was much lower under deficit irrigation regimes. Hence, the irrigation water productivity was higher in this regime as compared with the full irrigation conditions. The increased yields and irrigation water productivity achieved under deficit irrigation might be due

TABLE 6 Interaction of irrigation levels and mulching on fruit yield/plant of VNR Bihi of guava.

Irrigation levels	Mulching				
	Silver-black	Black	Organic	Without mulch	Mean
	2019–2020				
DI_{50}	33.40[e]	32.28[e]	31.76[e]	31.27[e]	32.18[c]
DI_{75}	49.10[a]	46.04[b]	42.55[cd]	40.35[d]	44.51[a]
FI_{100}	45.16[bc]	43.92[bc]	42.50[cd]	40.15[d]	42.93[b]
Mean	42.55[a]	40.75[b]	38.94[c]	37.26[d]	
	2020–2021				
DI_{50}	35.22[e]	34.38[e]	34.27[e]	32.44[e]	34.08[c]
DI_{75}	51.72[a]	47.10[b]	44.80[bcd]	42.93[cd]	46.64[a]
FI_{100}	46.76[b]	45.85[bc]	43.65[cd]	42.50[d]	44.69[b]
Mean	44.57[a]	42.44[b]	40.91[b]	39.29[c]	
	Pooled				
DI_{50}	34.31[e]	33.33[ef]	33.02[ef]	31.85[f]	33.13[c]
DI_{75}	50.41[a]	46.57[b]	43.68[c]	41.64[d]	45.57[a]
FI_{100}	45.96[b]	44.89[bc]	43.07[cd]	41.33[d]	43.81[b]
Mean	43.56[a]	41.59[b]	39.92[c]	38.27[d]	

DI_{50}, deficit irrigation at 50% ETc; $DI_{75,}$ deficit irrigation at 75% ETc; FI_{100}, full irrigation at 100% ETc. Values marked by a different letter differ significantly according to Duncan's multiple range test ($p \leq 0.05$).

to the excellent soil water relationship, with higher concentrations of oxygen present in the root zone and efficient utilization of water and nutrients. A deficit of or excessive water stress leads to stomatal closure, thereby improving the irrigation water productivity in water-stressed plants. An improvement in the irrigation water productivity in response to deficit irrigation compared with full irrigation was also reported in citrus (Pérez-Pérez et al., 2008; García-Tejero et al., 2010a), pomegranates (Dinc et al., 2018), mangos (Upreti et al., 2018), and guava (Preet et al., 2021).

The irrigation water productivity was influenced by different types of mulches, with plants mulched using MSB exhibiting significantly higher irrigation water productivities, followed by MB, with the lowest productivity observed in MWM over both years of study (Figure 3D). Sakariya et al. (2018) reported a higher irrigation water productivity using silver-black mulch in the papaya variety Madhu Bindu in Taiwan. Consistent with the above findings, da Silva et al. (2009) reported similar findings in mangos and Tiwari et al. (2014) in sapota. The interactive relationship between deficit irrigation and mulch, i.e., DIxM, and its effect on the irrigation water productivity varied significantly in both consecutive years. Pooled data for the 2 years indicated that the irrigation water productivity improved by 47.28% in guava plants irrigated under DI50MSB as compared with FI100MWM.

3.6 Ascorbic acid (mg/100 g)

The data presented in Table 7 reveal that various irrigation levels significantly influenced the ascorbic acid content of guava during 2019–20 and 2020–21. Plants irrigated at the DI75 (deficit irrigation at 75% ETc) level exhibited a maximum ascorbic acid content of 112.87 mg/100 g, followed by 102.85 mg/100 g for DI50 (deficit irrigation at 50% ETc), with a minimum recorded (90.41 mg/100 g) for FI conditions (full irrigation at 100% ETc). Similarly, during 2020–21, the maximum ascorbic acid concentration (115.44 mg/100 g) was recorded for DI75 (deficit irrigation at 75% ETc), and the minimum (93.30 mg/100 g) for FI (full irrigation at 100% ETc). In our study, the water deficit increased the vitamin C content of the fruit as compared with full irrigation. The tolerance to water deficit is correlated with ascorbic acid accumulation, which plays an important role in ROS (reactive oxygen species) detoxification (Wang et al., 2012). However, vitamin C is a major antioxidant in plants, capable of neutralizing active forms of oxygen. These results were

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TABLE 7 Ascorbic acid content of VNR Bihi guava influenced by irrigation levels and mulching.

Treatments	Ascorbic acid (mg/100 g)		
	2019–2020	2020–2021	Pooled
(A) Irrigation levels			
DI50	102.85[b]	105.50[b]	104.17[b]
DI75	112.87[a]	115.44[a]	114.16[a]
FI100	90.41[c]	93.30[c]	91.85[c]
(B) Mulching			
Silver-black	105.74[a]	109.03[a]	107.39[a]
Black	103.61[a]	106.53[a]	105.07[b]
Organic	100.59[b]	103.03[b]	101.81[c]
Without mulch	98.23[b]	100.40[b]	99.32[d]
Interaction (A × B)	s	s	s

similar to those of Ripoll et al. (2016), who found that water stress during the maturation phase increased the vitamin C levels. This effect could be related to the overall attempt made by the tree to combat water stress *via* the *de novo* synthesis of ascorbic acid (Navarro et al., 2010). Kowitcharoen et al. (2018) also suggested that increases in ascorbic acid contents in water deficit trees bearing sugar apple fruits may have been caused by abiotic stress conditions. Normally, stress conditions can induce ABA biosynthesis, which can promote hydrogen peroxide production; hydrogen peroxide is classified as a type of stress signal that may induce the antioxidant system in the plant to maintain or increase the ascorbic acid content. Singh et al. (2015) and Gupta (2019) also reported similar findings for guava and litchi, respectively, with higher ascorbic acid contents observed at a mild water deficit condition as compared with full irrigation.

In the year 2019–20, the highest ascorbic acid content was 105.74 mg/100 g, recorded in plants mulched using silver-black, followed by plants mulched with black (103.61 mg/100 g), and the lowest value (98.23 mg/100 g) observed for MWM (without mulch). During the second year of the study, the highest ascorbic acid content (109.03 mg/100 g) was also observed for MSB (silver-black) mulch, and the lowest (100.40 mg/100 g) for MWM (without mulch). However, MSB and MB were statistically at par with each other in both years of the study (Table 7). The increase of the ascorbic acid contents in guava fruits under different mulches might be due to optimum soil moisture content, providing excellent fruit quality parameters and soil nutrient status throughout the experimentation period (Tiwari et al., 2014). Prakash et al. (2011) and Singh (2020) also reported significant increases in the ascorbic acid contents under various mulches, for mango and guava, respectively. Furthermore, the interactive effect of deficit irrigation and mulching (DIxM) indicated that the ascorbic acid content of guava fruit was statistically significant, as mentioned in Table 7. A maximum pooled value for the ascorbic acid content was obtained: 116.22 mg/100 g for DI75MSB (deficit irrigation at 75% ETc and using silver-black mulch), which was significantly higher than other treatments. Similar findings were also observed by Joshi et al. (2012), who reported that drip irrigation coupled with mulch application significantly improved the ascorbic acid content in litchi fruits.

3.7 Correlation matrix

The degree of linear association of the fruit yield with all other variables (plant height, canopy spread, canopy volume, stem girth, leaf area, number of fruits, fruit weight, fruit length, fruit diameter, RLWC, proline content, chlorophyll, leaf macronutrients and micronutrients, and IWP) is presented in a correlation matrix in Table 8 and in Figure 4. The yield positively correlated with the total chlorophyll content, leaf area, leaf P content, canopy volume, canopy spread, fruit diameter, plant height, RLWC, fruit weight, leaf Cu content, fruit length, number of fruits, and the leaf K and N contents, while significant negative correlations were observed with the IWP and the leaf proline content.

3.8 Stepwise regression analysis

Stepwise regression analysis was performed by using the fruit yield (kg/plant) as a dependent variable and the remaining variables as independent variables. The correlation matrix (Table 8) showed a significant correlation among independent variables, which generates a multicollinearity problem. Stepwise regression analysis overcomes the problem of multicollinearity. The results revealed that out of the 20 independent variables, four (leaf proline content, fruit weight, irrigation water productivity, and leaf Cu content) were considered to explain the variable fruit yield. The regression model was found to be highly significant, with F calculated to be 157.9 (p-value = 2.2 × 10–16). The regression coefficients for all variables are shown in Table 9. The four variables leaf proline content, fruit weight, IWP, and leaf Cu content were found to be significant, and can be used to predict the fruit yield. The regression model is as follows:

$$\text{Fruit yield} = 34.04 - 0.65 \times \text{leaf proline} - 0.64 \times \text{leaf Cu} + 0.21 \times \text{IWP} + 0.05 \times \text{fruit weight} \quad (9b)$$

The R^2 value was 0.904, which means that 90.40% of the variation in the dependent variable (fruit yield) is explained by the model. The adjusted R^2 value was 0.898. The low values for goodness of fit statistics such as the RMSE (1.91), MAE (1.52), and MAPE (3.83) indicate that there is a small deviation between

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TABLE 8 Pearson's correlation matrix for plant-based observation in VNR Bihi guava.

	YLD	PH	CS	CV	SG	LA	NF	FW	FL	FD	RLWC	PRL	CHL	N	P	K	CU	MN	ZN	FE	IWP
YLD	1																				
PH	0.69**	1																			
CS	0.7**	0.77**	1																		
CV	0.71**	0.8**	0.94**	1																	
SG	0.58**	0.64**	0.75**	0.82**	1																
LA	0.75**	0.67**	0.67**	0.68**	0.62**	1															
NF	0.59**	0.22NS	0.04NS	0.07NS	0.11NS	0.49**	1														
FW	0.68**	0.69**	0.87**	0.88**	0.65**	0.45**	−0.03NS	1													
FL	0.63**	0.69**	0.88**	0.91**	0.71**	0.59**	0.02NS	0.85**	1												
FD	0.69**	0.75**	0.88**	0.89**	0.71**	0.63**	0.13NS	0.81**	0.83**	1											
RLWC	0.69**	0.62**	0.59**	0.6**	0.61**	0.74**	0.56**	0.28*	0.56**	0.58**	1										
PRL	−0.86**	−0.72**	−0.66**	−0.7**	−0.66**	−0.85**	−0.67**	−0.53**	−0.62**	−0.68**	−0.87**	1									
CHL	0.8**	0.7**	0.7**	0.74**	0.74**	0.86**	0.59**	0.51**	0.65**	0.69**	0.88**	−0.95**	1								
N	0.51**	0.62**	0.6**	0.7**	0.64**	0.53**	0.17NS	0.53**	0.62**	0.55**	0.5**	−0.63**	0.62**	1							
P	0.75**	0.68**	0.65**	0.69**	0.71**	0.74**	0.5**	0.51**	0.61**	0.64**	0.81**	−0.9**	0.87**	0.76**	1						
K	0.58**	0.68**	0.6**	0.67**	0.64**	0.76**	0.36**	0.41**	0.61**	0.62**	0.73**	−0.79**	0.8**	0.73**	0.84**	1					
CU	0.68**	0.72**	0.7**	0.74**	0.69**	0.75**	0.44**	0.54**	0.66**	0.69**	0.8**	−0.85**	0.87**	0.75**	0.91**	0.84**	1				
MN	0.48**	0.58**	0.55**	0.59**	0.59**	0.6**	0.29*	0.28*	0.45**	0.56**	0.63**	−0.66**	0.7**	0.61**	0.73**	0.72**	0.81**	1			
ZN	0.57**	0.61**	0.59**	0.63**	0.55**	0.73**	0.27*	0.29*	0.57**	0.58**	0.74**	−0.75**	0.79**	0.68**	0.8**	0.84**	0.85**	0.71**	1		
FE	0.53**	0.55**	0.54**	0.6**	0.58**	0.69**	0.45**	0.24*	0.57**	0.57**	0.72**	−0.74**	0.77**	0.68**	0.77**	0.81**	0.82**	0.76**	0.82**	1	
IWP	−0.32**	−0.49**	−0.62**	−0.63**	−0.49**	−0.47**	0.08NS	−0.52**	−0.62**	−0.63**	−0.5**	0.54**	−0.49**	−0.52**	−0.54**	−0.63**	−0.56**	−0.46**	−0.51**	−0.53**	1

YLD, yield; PH, plant height; CS, canopy spread; CV, canopy volume; SG, stem girth; LA; leaf area; NF, number of fruits; FW, fruit weight; FL, fruit length; FD, fruit diameter; RLWC, relative leaf water content; PRL, proline; CHL, total chlorophyll; N, nitrogen; P,Phosphorus; K, potassium; CU, copper; MN, manganese; ZN, zinc; FE, iron; IWP, Irrigation water productivity. Correlation values followed by * indicates the significance of correlation at $p < 5\%$ probability level and correlation values followed by ** indicate significance at $p < 1\%$; NS: not significant.

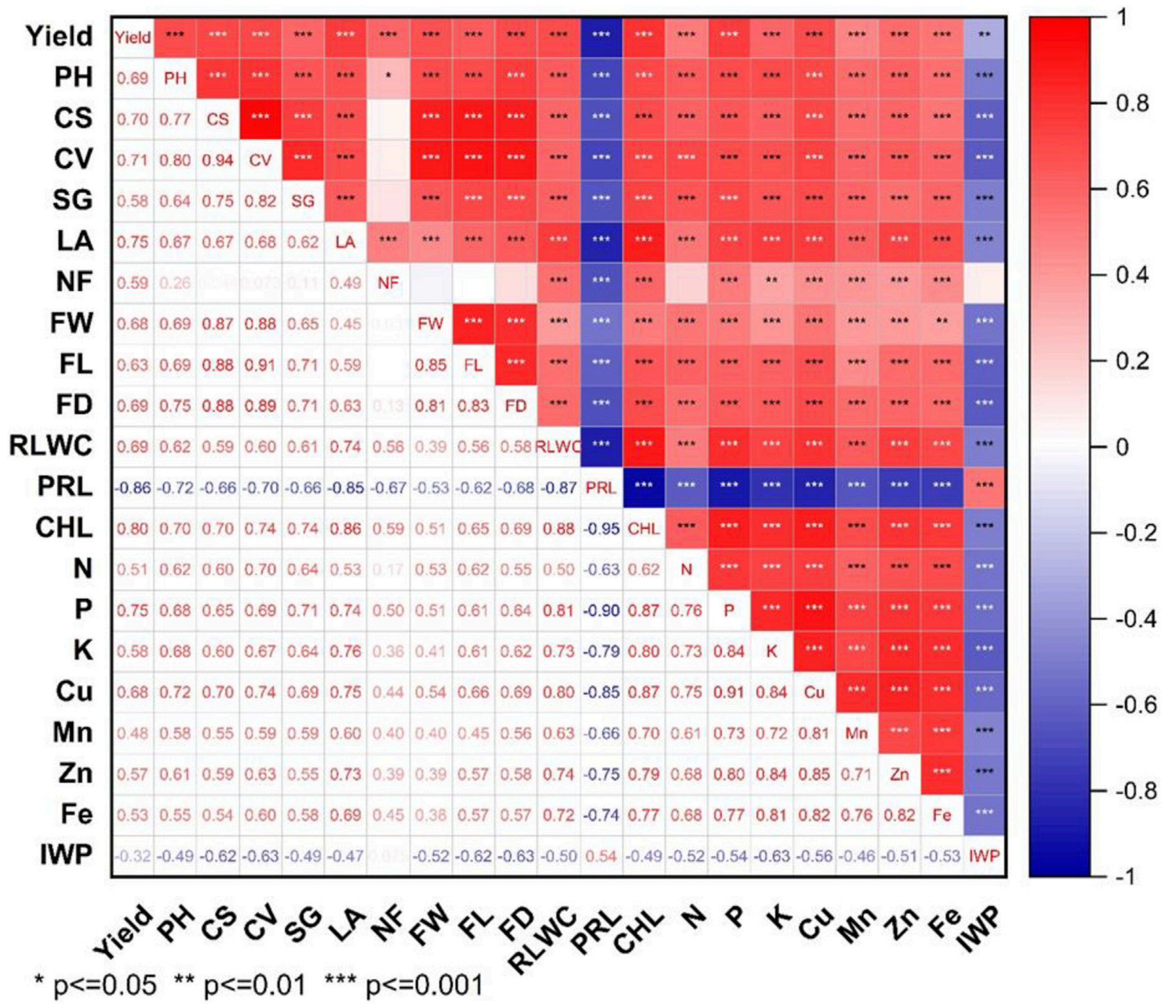

FIGURE 4
Pearson's correlation matrix for plant-based observation in VNR Bihi guava.

the actual values and the predicted values, as shown in Figure 5A. The run test statistic of the residuals was 37 with a p-value of 0.99, indicating that the residuals are random and do not follow any pattern. The Shapiro–Wilk test statistic was 0.99, with a p-value of 0.88, indicating that the residuals follow a normal distribution. Thus, residual analysis indicates that the regression model does not violate the assumption of normality or the randomness of the residuals.

3.9 Principal component regression analysis

PCA was performed for the 20 variables, and three principal components showing eigen values over one were used to predict the fruit yield. The results showed that the first three principal components captured 83.25% of the variation of the data set. The eigen values and the percentage of variance, explained by the principal component, is shown in Table 10. The multiple linear regression model was fitted using the three principal components as dependent variables and the yield as the independent variable.

The principal component regression model was found to be highly significant, with F calculated to be 125.1 (p-value = 2.2×10^{-16}). The regression coefficients for all variables are shown in Table 11. Among the three principal components, PC_1 and PC_3 exhibited significant regression coefficients. The regression model is as follows:

$$\text{Fruit yield} = 40.83 + 1.34 \times PC_1 - 0.27 \times PC_2 + 2.99 \times PC_3 \tag{10}$$

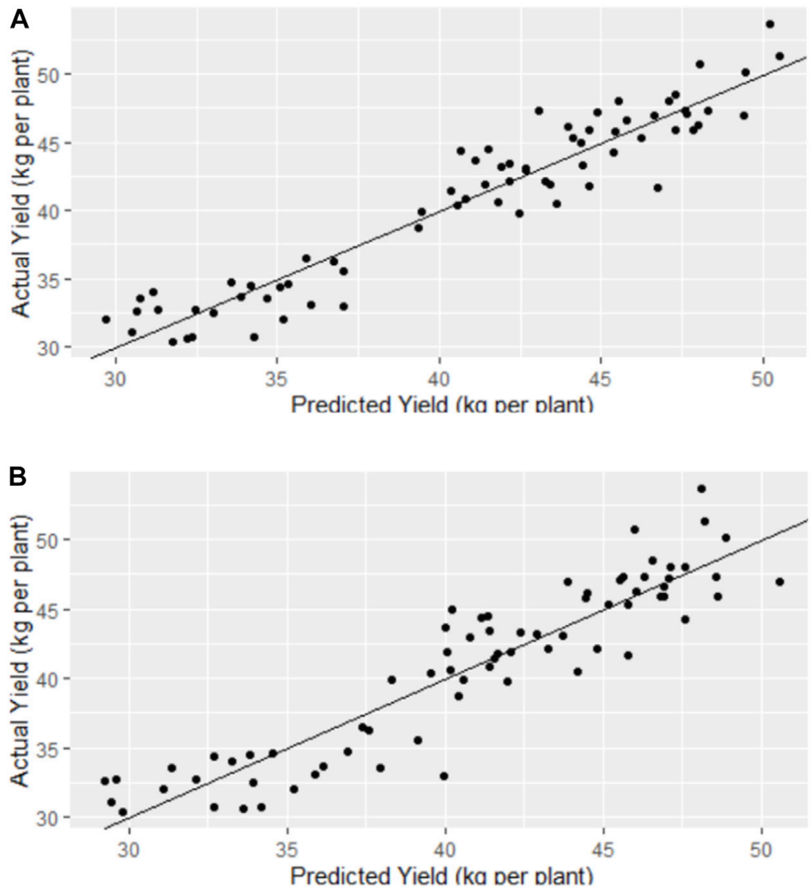

FIGURE 5
Actual yield vs. predicted yield of VNR Bihi guava plants by **(A)** step wise regression and **(B)** principal component regression.

TABLE 9 Regression coefficient estimates for different variables in step wise regression analysis.

Variable	Regression coefficient estimate
Intercept	34.04**
Leaf proline content	−0.65**
Fruit weight	0.05**
Irrigation water productivity	0.21**
Leaf copper content	−0.64**

"** Indicates significance level at p < 0.01."

The R^2 and adjusted R^2 values were 0.846 and 0.839, respectively. The lower values of goodness of fit statistics such as the RMSE (2.41), MAE (1.93), and MAPE (4.87) indicate that there is a small deviation between the actual values and the predicted values, as shown in Figure 5B. The run test statistic of the residuals was 24 with a *p*-value of 0.002, indicating that the residuals are not random. The Shapiro–Wilk test statistic was 0.98, with a *p*-value of 0.78, indicating that the residuals follow normal distributions. Thus, residual analysis indicates that the regression model does not violate the assumption of normality and does violate the randomness of the residuals.

TABLE 10 Principal components for plant-based variables with eigen values.

Principal component	Eigen value	Proportion of variance	Cumulative variance
PC1	3.65	0.66	0.66
PC2	1.53	0.11	0.78
PC3	1.03	0.04	0.83

PC1, Principal component 1; PC2, Principal component 2; PC3 Principal component 3.

TABLE 11 Regression coefficient estimates of principal components.

Variable	Regression coefficient estimate
Intercept	40.83**
PC1	1.34**
PC2	−0.27^{NS}
PC3	2.99**

PC1, Principal component 1; PC2, Principal component 2; PC3 Principal component 3.
"** Indicates significance level at $p < 0.01$ NS indicates non significant"

4 Conclusion

Fully-irrigated plants and silver-black mulch produced the highest vegetative growths and leaf nutrient contents. However, deficit irrigation at 75% Et_c, along with silver-black mulch used during the fruit growth period produced higher numbers of fruits per plant, higher average fruit weights, and higher fruit yields. Moreover, the irrigation water productivity also improved substantially in deficit irrigated plants. Out of both the techniques used for the prediction of fruit yield, the stepwise regression model presented a higher value of the adjusted R^2 and lower values of the RMSE, MAE, and MAPE. Furthermore, the stepwise regression model also follows the assumptions of normality and randomness of the residuals. Thus, we conclude that the stepwise regression model is preferred over the principal component regression model to predict the fruit yield in this study, using variables *viz.*, the leaf proline content, fruit weight, IWP, and leaf Cu content. Based on the present findings, it can be inferred that application of deficit irrigation at 75% ET_c using silver-black mulch imposed desirable levels of water stress on guava plants. This improved their irrigation water productivities, yields, and the fruits quality, and could be a superior option for guava cultivation in the subtropical, humid Tarai conditions of Uttarakhand, India, as well as in regions with similar agro-climatic conditions.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

Conceptualization, RJ, VS, and PS; methodology, DV; AC; software, RP; validation, RJ, VS, and PS; formal analysis, RP; investigation, SJ; resources, VS; data curation, RP; writing—original draft preparation, RJ, VS; writing—review and editing, NA-A and SA; visualization, RJ; supervision, VS; project administration, AC; funding acquisition, MA-S. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Combined evaluation of corporate ecological and environmental responsibility: Evidence for forest preservation from Chinese forestry companies

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The ecological benefit of forest has an important influence on the sustainable development of society, thus, forest management has become a critical strategic action. Forest preservation is an inclusive process which depends on collaboration among a wide range of stakeholders. Forestry companies, who own and manage forest resources, are responsible for forest preservation and ecological construction, which is called corporate ecological environmental responsibility (CEER). Most existing analyses, however, were limited to corporate environmental responsibility (CER) and ignored the ecological responsibility of forestry enterprises. Therefore, in order to better play the role of forestry companies in forest preservation, it is urgent to define the content and the measurement of CEER. This paper established a CEER index system based on the characteristics of forestry enterprises. Furthermore, evaluated the CEER level of forestry enterprises using the combined evaluation method based on the GINI criterion, which is more effective and reasonable. It is found that forestry ecological environmental responsibility emphasizes ecological improvement and has shifted from traditional environmental protection to ecological construction. Qingshan Paper, Sun Paper, and Yong'an Forestry perform the best in CEER among all forestry companies. In addition, the results showed a low level but an obvious upward trend in forestry CEER and a noticeable heterogeneity in the performance of CEER in different forestry industries. Our findings can be useful for further promoting the ecological benefits of forest companies and developing relevant policies.

KEYWORDS

forest preservation, corporate ecological environmental responsibility, combined evaluation, forestry companies, carbon neutrality

1. Introduction

With the deepening of green development, people pay more attention to ecological environmental protection. China has actively promoted green development and pledged to achieve carbon neutrality by 2060 to address climate change. Forests are the earth's lungs, the largest carbon storage and oxygen generator on land. Forest carbon sequestration is one of the most cost-effective ways to address climate change. China's total forest carbon storage has reached 9.2 billion tons and is still increasing yearly. In the context of carbon neutrality, the role of forestry becomes even more prominent (Wang et al., 2022). Forest is crucial for maintaining climate and ecological security and has been promoted to a strategic height concerning human survival and development, future, and destiny. Benefiting from the right to manage forest resources, forestry enterprises are a particular organization that can obtain economic, social, and ecological benefits from their management activities (Sui and Zhang, 2012). Thus, due to the ownership and management of forest resources, enterprises in the forestry industry should combine their advantages in green resources, give full play to its role in forest preservation, and actively fulfill ecological environmental responsibilities.

Achieving carbon neutrality requires joint efforts, of which "emission reduction" and "carbon sequestration" are the two main approaches. "Emission reduction" refers to the industrial sector's efforts to improve resource efficiency and energy consumption to reduce carbon dioxide emissions. "Carbon sequestration" is to increase the absorption of carbon dioxide by protecting forests, grasslands, and wetlands (Chen et al., 2022). Common enterprises mainly manage climate change through "emission reduction." In contrast, forestry enterprises can do it through "carbon sequestration" in addition to "emission reduction." Therefore, forestry enterprises also bear the responsibility of forestry ecological construction other than environmental protection for common enterprises. It should be noted that environmental protection and forestry ecological construction are two different measures. The former is to reduce the negative externality of business activities by reducing pollution and environmental damage. However, the latter requires further improvement in the ecological environment to increase the positive externality of forestry management (Zhang, 2021). Thus, forestry enterprises shoulder a special mission in ecological construction. The responsibilities of forestry enterprises for environmental and forest preservation should include green procurement policies, sustainable forest activities, ecological efficiency improvement, and renewable materials, emphasizing the impact on forest and ecosystem services (D'Amato et al., 2015).

It is normal to refer to the responsibility of environmental protection when discussing corporate environmental responsibility. However, its content is mainly limited to energy conservation, emission reduction, and clean production, ignoring the ecological responsibility of forest resources protection and ecological restoration of forestry enterprises. To be precise, forestry enterprises' environmental responsibility should be called "corporate ecological environmental responsibility (CEER)" rather than a simple "corporate environmental responsibility (CER)." In order to better play the role of forestry enterprises in forest preservation and ecological environment protection, questions such as the content of forestry CEER and how to measure and evaluate it deserves urgent discussion.

The research analyzed the content of CEER based on the particularity of forestry enterprises, constructed the CEER evaluation index system based on the natural resource-based view, and then evaluated the forestry CEER using a more robust combined evaluation method. Possible contributions of this paper are: (1) The research focused on a particular environmental responsibility of forestry enterprises, namely ecological environmental responsibility. Based on the characteristics of the forestry industry, a CEER index system was constructed, which enriched the existing research results. (2) A new evaluation method, the combined evaluation method based on the GINI criterion, was adopted to evaluate the ecological environmental responsibility of forestry enterprises, which avoided the data fluctuation caused by a single evaluation method, and made the evaluation more effective and reasonable.

The remainder of this study is arranged as follows. Section 2 is devoted to the literature review. Section 3 elaborates on the research method. Section 4 describes the empirical research design, including data source and index construction. Section 5 outlines the empirical results. Section 6 shows the further discussion of the empirical results, while Section 7 presents the conclusions and limitations.

2. Literature review

2.1. Definition of environmental responsibility

Our environment largely depends on the exploitation of natural resources by companies. Their operation often leads to air pollution, water pollution, soil loss, and other environmental issues. As a result, companies must undertake social responsibilities because the environment is a public resource. To improve environmental sustainability, companies must be responsible for environmental problems and completely disclose environmental information in financial statements in an accurate, timely manner (Long et al., 2022). The rollout of the concept of CER has emphasized the environmental responsibilities undertaken by all companies (Cohen et al., 2013). However, researchers seem to be more concerned about the environmental responsibility of polluting companies. CER is defined as any precautions and policies corporations apply to reduce environmental damage (Sarmento et al., 2005). Carroll (1979) believes that environmental responsibility is a part of social responsibility. Corporate social responsibility (CSR) can be divided into economic, legal, ethical, and conscious responsibility, while environmental protection, charitable donation, and employment support are involved in conscious responsibility. Enderle and Tavis (1998) proposed a corporation concept that is based on the responsibility to balance economic, social, and environmental responsibilities. Some scholars define CER as the environmentally friendly behavior of enterprises beyond the requirements of laws and regulations (Lyon and Maxwell, 2008). The broader definition of CER refers to the companies' behavior in managing the relationship between business activities and the natural environment (Aragón-Correa and Sharma, 2003).

As for forestry enterprises, most existing literature focuses on their social responsibility, and only a handful of research directly focuses on environmental responsibility. For example, Li and Gao (2019) found that forestry CSR is closely related to company scale, industry, ownership, and forest resources. Lu et al. (2017) found that the CSR of forestry enterprises was positively correlated with firm size and equity concentration but had no significant relation with profitability and financial leverage. However, from the forestry CSR priority, the main content of forestry CSR emphasizes environmental responsibility rather than social responsibility due to its direct and high impact on the natural environment (Nowak, 2006). Even though forestry enterprises fulfilled their social responsibilities, their social influences are often overlooked due to their environmental impacts (Kärnä et al., 2003). The focus of forestry CSR lies not only on contributing to social resource redistribution but also on balancing the relationship between profit gains and the sustainable development of the ecological environment (Toppinen and Korhonen-Kurki, 2013). Li and Toppinen (2011) believe that forestry enterprises would be selective in social responsibility content to report, with more environmental indicators than economic or social ones. Vidal and Kozak (2008) analyzed the social responsibility content of forestry companies and found that the most frequently mentioned responsibility was sustainable forest management, followed by accounting, employment, recycling, and forest. Although many scholars are aware of the importance of the environmental responsibility of forestry enterprises, few directly study it.

2.2. Evaluation of environmental responsibility

The existing evaluation methods of CER or CSR are divided into two categories. The first is the content analysis method, which assigns values according to the information disclosed to evaluate the enterprise's environmental (social) responsibility level. Li and Gao (2019) applied this method to evaluate the social responsibility of forestry enterprises. To evaluate environmental responsibility, he designed 10 contents, including pollution control, environmental restoration, recycling, and environmental products (Vidal and Kozak, 2008). Content analysis can transform qualitative information into quantitative data for analysis. However, it could be limited by the information disclosure, as the responsibility might be fulfilled but not yet disclosed.

Another evaluation is index analysis, constructing indexes for quantitative analysis. To meet two policy objectives of environmental health and ecosystem vitality, Pinar (2022) set up 32 environmental performance indicators, including climate change, pollution emission, and waste management. Li et al. (2021) proposed a time-based entropy method that can evaluate long-term changes in CSR and consider a company's static and dynamic aspects. Regarding the environmental responsibility of CSR, "urban maintenance and construction tax" was chosen as the measurement index for analysis (Li et al., 2021). Wang Y. L. et al. (2020) selected energy saving and intelligent operation management, water resource management, waste management, and air pollution prevention to measure the environmental responsibility of forestry enterprises. Index analysis requires objective comprehensiveness of indicators and data availability.

Index analysis has a major problem, the balance of index weight. Two main methods for assigning index weight are the subjective and the objective weighting methods. The typical subjective methods include the analytic hierarchy process, comprehensive scoring, and fuzzy evaluation method. Li et al. (2020) proposed an improved analytic hierarchy process-back propagation (AHP-BP) neural network algorithm to evaluate companies' CSR performance, and results showed that this improved AHP-BP neural network model could effectively estimate CSR performance. Ni et al. (2019) established a green index system and quantitatively evaluated the environmental quality of Guizhou geological parks based on the fuzzy comprehensive evaluation system. The subjective method can determine the importance of the evaluation index according to expert experience. However, human factors greatly influence the weight and cannot objectively reflect the index data information.

The objective weighting methods determine the weight of indexes by comparing the data information of different evaluation objects, which is not affected by human factors. The evaluation results are more objectively compared with subjective methods. Therefore, it has been extensively used at present. Common objective weighting methods include the entropy weight method, the technique for order preference by similarity to an ideal solution (TOPSIS) method, the coefficient of variation (CV) method, the criteria importance through intercriteria correlation (CRITIC) method, and the structural equation model. Considering the heterogeneity of stakeholders in the fuzzy environment, Yi et al. (2022) established a TOPSIS evaluation framework based on an internal type-2 trapezoidal fuzzy numbers (IT2TrFN) analytical hierarchy process. The author later evaluated the CSR performance of listed companies in 2017–2018. Aiming to investigate the employees' recognition level of CSR in companies, Stojanović et al. (2021) set CSR criteria indicators through five dimensions and determined the criteria weights using the entropy method. Yalcin and Ünlü (2018) evaluated the performance of initial public offering (IPO) firms using multiple measures and then used the CRITIC method to evaluate and rank IPOs' performances.

However, different evaluation methods are based on different principles and require different information, which may lead to different evaluation results. Some scholars put forward the idea of a combined evaluation. Wang W. et al. (2020) insisted that single method evaluation will lead to inconsistent results and proposed a combined evaluation method based on seven individual methods to assess the risk of comprehensive urban disasters. When evaluating the benefit of transnational power networking projects, Zhao et al. (2019) combined the order relation method and the GINI coefficient method to synthesize subjective and objective information. Fang and Song (2019) proposed an objective combined evaluation method based on the GINI criterion, combining five single evaluation methods, such as the entropy weight method and TOPSIS, using their results to evaluate the technological innovation ability of enterprises.

2.3. Literature summary

It can be seen that the current research on environmental responsibility has made some progress, but there are still limitations:

From the perspective of environmental responsibility content, almost all existing studies implicitly regard enterprises as "the source of pollution" (Liu et al., 2021; Jiang et al., 2022). Thus the CER content is tied up with "pollution mitigation" (Yang, 2012), and the study object focuses on heavy pollution industries with negative externalities, such as steel, food processing, and mineral industries(Kovalevsky et al., 2018; Chen et al., 2020). Forestry is a particular industry that integrates economic, ecological and social benefits. As Senko pointed out, day-to-day forestry operations may have significant implications for the sustainable development of forests (Senko and Pykäläinen, 2020), so their environmental responsibility must be different from ordinary enterprises (Sharma and Henriques, 2005). Unfortunately, the existing research failed to pay attention to the positive environmental externalities of forestry enterprises, making the definition of CER less comprehensive.

In terms of environmental responsibility evaluation methods, on the one hand, the content analysis method was frequently used, while the index analysis was less used. However, the content analysis method does not apply to CER because the level of CER disclosure is generally low in China (Lu and Abeysekera, 2014). In addition, there is a discrepancy between the disclosure statements and the environmental performance of enterprises. Thus, the environmental responsibility level cannot be measured by the content of corporate disclosure alone (Acar and Temiz, 2020). On the other hand, the objective weighting method is widely used when adopting the index analysis method. However, previous literature used a single method to distribute each index's weight. Different evaluation mechanisms make the evaluation results of different methods on the same problem biased (Xu et al., 2019). The combined evaluation method can fully use more information and has stronger stability because the data fluctuation caused by a single method is smoothed (Zhang et al., 2016). It is verified that the combination idea can obtain high convergence and credibility evaluation results and solve the problem of inconsistent evaluation results of different methods (Li et al., 2018). Regrettably, the combined method has not been applied to CER.

In order to solve the above problems, this paper focused on the particularity of forestry enterprises, analyzed the content of CEER and constructed the CEER evaluation index system based on the natural resource-based view. By using a more robust combined evaluation method, this paper evaluated the CEER level of forestry enterprises and found an upward trend in forestry CEER and a noticeable heterogeneity in the performance of CEER in different forestry industries.

3. Research method

Given the different importance of each indicator, different weights should be given to these specific indicators when constructing the index system to evaluate the forestry CEER. Considering that the subjective weighting method is highly arbitrary as it depends on personal experiences, the objective weighting method was chosen according to the conventional practice. In this paper, the combined evaluation method was adopted to avoid the deviating results caused by the abnormal weight of individual indicators with a single method. The basic idea of the combined evaluation is to combine the results of single methods with appropriate ways to obtain the integrated value. Fang and Song (2019) proposed an objective combined evaluation method based on the GINI criterion, and it is more effective than other combined evaluation methods, including the average value, Borda, and Copeland. In this paper, the method was applied to evaluate CEER. Specifically, single evaluation methods such as entropy weight, CRITIC, and CV were first used to evaluate forestry enterprises' CEER. These single evaluation results were combined to obtain a completed evaluation result of CEER by applying the combined evaluation method based on the GINI criterion.

3.1. Single evaluation methods

Suppose there are m sample enterprises and n evaluation indexes, X_{ij} is the value of index j of enterprise i, and the original matrix is $A = (x_{ij})_{m \times n}$. In order to eliminate the influence of different measurements on evaluation results, it is necessary to standardize each index and get a standardized matrix $A = (y_{ij})_{m \times n}$, the maximum value of the standardized index is 1, and the minimum value is 0.

Standardization formula of positive index

$$y_{ij} = \frac{x_{ij} - x_j^{\min}}{x_j^{\max} - x_j^{\min}} \quad (1)$$

Standardization formula of negative index

$$y_{ij} = \frac{x_j^{\max} - x_{ij}}{x_j^{\max} - x_j^{\min}} \quad (2)$$

Where, x_{ij} represents the index j's value of enterprise i, represents the maximum value of index j, and represents the minimum value of index j.

3.1.1. Entropy weight method

The concept of entropy originated from thermodynamics and was later introduced into information theory. Information entropy can reflect the degree of indicators' variation, thereby making a comprehensive evaluation. The greater the gap between the indicators, the greater the information provided by the indicator, and the greater the role it plays in the comprehensive evaluation, so does the weight. Otherwise, the smaller the weight. The calculation of entropy weight is usually divided into the following steps:

(1) Calculate the relative proportion, denoted as p_{ij};

$$p_{ij} = y_{ij} \div \sum_{i=1}^{m} y_{ij} \quad (3)$$

Where, y_{ij} is the standardized value of index j of enterprise i, and m is the number of sample enterprises.

(2) Calculate the entropy value of index j, denoted as E_j;

$$E_j = -\frac{1}{\ln m} \sum_{i=1}^{m} p_{ij} \ln p_{ij} \quad (4)$$

Where, p_{ij} is the relative proportion of the enterprise i in index j.

(3) Calculate the entropy weight of each index, denoted as w_j;

$$w_j = \frac{1 - E_j}{n - \sum_{j=1}^{n} E_j} \quad (5)$$

Where, E_j is the entropy value of index j, and n is the total number of evaluation indexes.

3.1.2. CRITIC method

CRITIC (criteria importance through intercriteria correlation) method is an objective weighting method proposed by Diakoulaki et al. (1995). The core idea of CRITIC lies in two indicators, volatility (contrast intensity) and conflict (correlation). The volatility is represented by standard deviation; the larger the standard deviation of the data, the greater the fluctuation and the higher the weight. The conflict is represented by the correlation coefficient; the larger the correlation value between indicators, the smaller the conflict, and the lower the weight. The calculation steps of CRITIC weight are as follows:

(1) Calculate the volatility of index j, denoted as S_j;

$$S_j = \sqrt{\frac{\sum_{i=1}^{m} \left(y_{ij} - \overline{y}_j\right)^2}{m - 1}} \quad (6)$$

Where, $\overline{y}_j$ is the mean of index j of all enterprises.

(2) Calculate the conflict of index j, denoted as A_j;

$$A_j = \sum_{k=1}^{n} \left(1 - r_{kj}\right) \quad (7)$$

Where r_{kj} represents the correlation coefficient between index k and index j.

(3) Calculate the information content of index j, denoted as C_j;

$$C_j = S_j \times A_j \quad (8)$$

Where, S_j is the volatility of index j, and A_j is the conflict of index j.

(4) Calculate the weight of index j, denoted as w_j;

$$w_j = \frac{C_j}{\sum_{j=1}^{n} C_j} \quad (9)$$

Where, C_j is the information content of index j.

3.1.3. CV method

The CV method assigns weight to each index according to the degree of variation of each index. A significant index CV indicates that the index has rich discriminative information and can clearly distinguish the evaluated objects, so the index should be assigned a greater weight. Otherwise, the index obtains a smaller weight. The CV is calculated by the mean and standard deviation of the index, and the calculation of weight is as follows:

(1) Calculate the CV of the evaluation index j, denoted as v_j;

$$v_j = \frac{S_j}{\overline{\overline{y}}_j} \quad (10)$$

Where, S_j is the standard deviation of the index j, $\overline{y}_j$ is the mean of index j.

(2) Calculate the weight of each indicator, denoted as w_j;

$$w_j = \frac{v_j}{\sum_{j=1}^{n} v_j} \quad (11)$$

Where, v_j is the CV of index j.

After obtaining the index weight of a single evaluation method, the evaluation result of the i[th] enterprise can be calculated as follows:

$$Y_i = \sum_{j=1}^{n} w_j y_{ij} \quad (12)$$

Where, w_j is the weight of index j, and y_{ij} is the standardized value of index j of enterprise i.

3.2. Combined evaluation based on GINI criterion

Fang and Song (2019) proposed an objective combined evaluation method based on the GINI criterion, which measures the information purity of different evaluation methods through the GINI coefficient. Then it assigns weights to different evaluation methods according to the degree of information purity. The greater the information purity of a certain evaluation method, the greater the certainty of the evaluation result of it, and the greater the weight is given to it. On the contrary, a smaller weight would be given. The specific calculation steps are as follows:

(1) Select K single evaluation methods to form a single method set;
(2) Use Kendall test to check whether the single method set is consistent. If not, re-select single methods to replace;
(3) Calculate the information purity of a single method k, denoted as d_k;

$$d_k = \sum_{i=1}^{m} p_{ik}^2 \quad (13)$$

Where, p_{ik} is the relative proportion, and the calculation formula is:

$$p_{ik} = Y_{ik} / \sum_{i=1}^{m} Y_{ik} \quad (14)$$

Where Y_{ik} is the evaluation result of the i[th] enterprise in the k[th] evaluation method, m is the total number of enterprises;

(4) Determine the weight of each single method, denoted as w_k;

$$w_k = d_k / \sum_{k=1}^{K} d_k \quad (15)$$

Where, d_k is the information purity of the evaluation method k;

(5) Calculate the combined evaluation value;

$$Z_i = \sum_{k=1}^{K} w_k Y_{ik} \quad (16)$$

Where, w_k is the weight of the evaluation method and Y_{ik} is the evaluation value of enterprise i of evaluation method k.

4. Empirical research

4.1. Sample selection and data sources

According to the Classification of Forestry and Related Industries (Trial), companies that do not have forest resources but carry out the follow-up processing of forest products also belong to forestry enterprises. Although these companies statistically belong to forestry enterprises, they have no forest resources and have the same environmental responsibility as general manufacturing companies. Therefore, this paper does not take them as research objects. The samples in this paper are A-share listed forestry companies with forest resources, including forest enterprises, forest-board integration enterprises, and forest-paper integration enterprises. Considering that the Implementation Rules of China's Forest Certification were promulgated in 2009, this paper chose 2009–2021 as the research period. This paper removed enterprises under Special Treatment in accordance with practice. For enterprises with industry changes during the study period, this paper only kept the data when the companies were in the forestry industry and deleted the rest data. As a result, 215 imbalanced panel data from 18 sample enterprises were obtained. Data for environmental investment, environmental penalties and biological assets come from the CSMAR database, data for environmental statement, non-commercial forest and forest land come from enterprises' annual reports and official websites, and data for environmental certification, environmental label product certification and forest certification come from the State Administration of Market Regulation website.

4.2. Construction of index system

CER is the strategic responsibility of CSR (Orazalin and Baydauletov, 2020). Based on the competitive advantage theory of the relationship between enterprises and the natural environment, Hart et al. put forward the natural resource-based view with three strategies: pollution prevention, product management, and sustainable development (Hart and Dowell, 2011). This paper constructed the forestry CEER index under the theoretical basis of the natural resource-based view.

Pollution prevention requires enterprises to minimize environmental pollution through source prevention, process control, and end treatment of clean production. This paper measured the responsibility of forestry enterprises in pollution prevention through two dimensions, environmental input and social relations.

The product management strategy requires enterprises to minimize exploiting non-renewable resources from the natural environment. According to the utilization of renewable resources, enterprises can modify existing products to reduce environmental pollution. Environmental management and green production were two dimensions chosen in this paper to measure the forestry enterprises' responsibility in product management.

The sustainable development strategy requires a long-term vision and avoid to pursue short-term profits that are harmful to the environment. Forest protection and ecological improvement were two dimensions chosen in this paper to measure the responsibility of forestry enterprises in sustainable development.

With the characteristics of forestry enterprises and data availability, nine indicators were designed under the six dimensions (as shown in Table 1). Among them, the index marked (+) represents a positive index, and the index marked (−) represents a negative index.

Table 2 shows the descriptive statistical results of each index. In pollution prevention strategy, significant differences between environmental investment and penalties, and about half of the enterprises have disclosed environmental responsibility reports. In product management strategy, 74% of the enterprises have obtained environmental certification, 30.2% have obtained environmental label product certification, and 41.9% have passed forest certification. In the sustainable development strategy, the forest land area owned by the sample enterprises was not much. In addition, the forest land area and biological assets value were significantly different, and the enterprises holding non-commercial forests account for only 17.2%.

5. Result and analysis

5.1. Forestry CEER results of single method

The mean score of forestry CEER using the entropy weight method, CRITIC method, and CV method is displayed in Table 3. By using these three methods, CEER obtained an average value of 0.286, 0.403, and 0.266, respectively. The data show great differences in the evaluation values of these three methods, resulting in different rankings, especially for Kangxin New Materials, Huatai Paper, Jilin Forest, and Fujian Jinsen Forestry. The Wilcoxon paired signed rank test was further used to compare the three methods. All hypotheses were rejected at the 1% level according to Table 4, which indicates the different results of the three single methods: median value of CRITIC (0.412) > median value of entropy weight (0.265) > median value of CV (0.250). Clearly, different single methods evaluate from different angles, and their results reflect the characteristics of a certain aspect, which is bound to lead to inconsistent conclusions. Therefore, it is necessary to combine different evaluation methods to smooth the volatility of a single method.

5.2. Forestry CEER results of combined evaluation

It is necessary to test whether the set of single methods is consistent before the combined evaluation. If it is consistent, the single methods can be combined; otherwise, new single methods need to be selected for combination. Kendall's W test was used to test the concordance of the three methods. According to Table 5, the Kendall coordination coefficient of the three evaluation methods is 0.964, and the null hypothesis is rejected at the 1% level, that is, the three single evaluation methods set is considered consistent and can be evaluated in combination.

TABLE 1 Index of forestry CEER.

Key strategy	Dimension	Index	Explanation
Pollution prevention	Environmental input	Environmental investment (+)	Amount of investment related to environmental protection
	Social relations	Environmental penalties (−)	Pollution discharge fees
		Environmental statement (+)	If the enterprise disclosed environmental statement, the value is 1; otherwise, the value is 0.
Product management	Environmental management	Environmental certification (+)	If the enterprise passed the ISO environment certification, the value is 1; otherwise, the value is 0.
	Green production	Environmental label product certification (+)	If the enterprise passed the environmental label product certification, the value is 1; otherwise, the value is 0.
		Forest certification (+)	If the enterprise passed the forest certification, the value is 1; otherwise, the value is 0.
Sustainable development	Forest protection	Forest land (+)	The area of forestland owned or controlled by the enterprise
	Ecological improvement	Biological assets (+)	Ending balance of biological assets
		Non-commercial forest (+)	If the enterprise owns non-commercial forest, the value is 1; otherwise, the value is 0.

TABLE 2 Data description of each index.

Key strategy	Dimension	Index	Min	Max	Avg	Std
Pollution prevention	Environmental input	Environmental investment	0.000	157730.755	3226.153	13300.38
	Social relations	Environmental penalties	0.000	14289.923	898.329	2484.173
		Environmental statement	0.000	1.000	0.433	0.497
Product management	Environmental management	Environmental certification	0.000	1.000	0.740	0.440
	Green production	Environmental label product certification	0.000	1.000	0.302	0.460
		Forest certification	0.000	1.000	0.419	0.494
Sustainable development	Forest protection	Forest land	0.060	1800.000	168.257	357.554
	Ecological improvement	Biological assets	0.000	899738.498	60584.437	106557.031
		Non-commercial forest	0.000	1.000	0.172	0.378

TABLE 3 Score of forestry CEER.

	Single method						Combined method	
	Entropy weight method		CRITIC method		CV method			
	Score	Rank	Score	Rank	Score	Rank	Score	Rank
Qingshan paper	0.486	1	0.578	2	0.431	1	0.498	1
Sun paper	0.419	3	0.604	1	0.371	3	0.462	2
Yong'an forestry	0.453	2	0.492	5	0.412	2	0.452	3
Kangxin new materials	0.403	4	0.41	9	0.363	4	0.383	4
Meili cloud	0.336	6	0.513	3	0.304	7	0.382	5
Huatai paper	0.317	9	0.511	4	0.298	9	0.373	6
Pingtan development	0.319	8	0.48	6	0.29	10	0.361	7
Jilin forest	0.326	7	0.394	11	0.363	4	0.360	8
Fujian Jinsen forestry	0.349	5	0.375	12	0.312	6	0.345	9
Yueyang paper	0.308	10	0.426	7	0.303	8	0.344	10
Bohui paper	0.278	11	0.413	8	0.244	11	0.310	11
Fenglin Wood	0.264	12	0.399	10	0.238	12	0.298	12
MYS group	0.213	13	0.356	13	0.198	14	0.254	13
Chenming paper	0.194	14	0.327	14	0.199	13	0.238	14
WeiHua	0.188	15	0.293	15	0.173	15	0.216	15
Tubao	0.137	16	0.256	16	0.13	16	0.172	16
Dare power Dekor	0.105	17	0.193	17	0.101	18	0.132	17
Yihua wood	0.092	18	0.184	18	0.105	17	0.126	18
Max	0.612		0.778		0.548		0.625	
Min	0.015		0.060		0.017		0.030	
Avg	0.286		0.403		0.266		0.274	
Std	0.154		0.177		0.134		0.152	

Next, the combined evaluation of the above three single methods was carried out following the steps in section 3.2. Table 6 shows the combination of the three methods. The information purity of the entropy weight method is 0.06, and the weight is 34.52%, indicating that the evaluation value of this method fluctuates the most, with the highest information purity and the best evaluation effect. The information purity of the CRITIC value is 0.055, and the weight is 31.92%, indicating that the value of this method fluctuates the least, with low certainty and a relatively poor evaluation effect. The information purity of the CV method is 0.058, and the weight is 33.56%, which is located between the former two.

Further, the weight of each index can be calculated. According to Table 7, among all dimensions, the most important one is ecological construction responsibility, accounting for 27.55%. That is because the forest resources owned by the forestry enterprises can fix a large amount of CO_2, protecting wetlands and woodland soil. In addition, forest carbon sequestration also benefits biodiversity protection and climate change. The second important is green production responsibility, accounting for 26.79%. As a fundamental market subject, forestry enterprises should perform adequately in green management during operation, such as recycling forest waste to improve the utilization

TABLE 4 Results of Wilcoxon analysis.

	Median (P25, P75)		Median difference	z-value	p
	Group 1	Group 2			
CRITIC *match* entropy weight	0.412(0.3,0.5)	0.265(0.2,0.4)	0.147	12.373	0.000***
CV *match* entropy weight	0.250(0.2,0.4)	0.265(0.2,0.4)	−0.014	8.878	0.000***
CRITIC *match* CV	0.412(0.3,0.5)	0.250(0.2,0.4)	0.162	12.587	0.000***

* $p<0.1$, ** $p<0.05$, *** $p<0.01$.

TABLE 5 Kendall W coordination coefficient analysis.

Evaluator	Evaluation object	Kendall coordination coefficient	χ^2	p
3	215	0.964	618.889	0.000***

* $p<0.1$, ** $p<0.05$, *** $p<0.01$.

TABLE 6 Combination of 3 single methods.

	Entropy weight method	CRITIC method	CV method
Information purity	0.06	0.055	0.058
Weight	34.52%	31.92%	33.56%

TABLE 7 Weight of each index.

Dimension	Weight	Index	Weight
Environmental input	10.94%	Environmental investment	10.94%
Social relations	14.86%	Environmental penalties	2.55%
		Environmental statement	12.31%
Environmental management	7.36%	Environmental certification	7.36%
Green production	26.79%	Environmental label product certification	14.76%
		Forest certification	12.03%
Forest protection	12.50%	Forest land	12.50%
Ecological improvement	27.55%	Biological assets	9.77%
		Non-commercial forest	17.78%

efficiency of forest resources, extensively use of other new environmental materials as raw materials to produce low carbon pollution-free products that meet the social demand for green products. In contrast, environmental management and environmental input were less weighted, accounting for 7.36 and 10.94%, respectively. The reason is that environmental pollution prevention has become a continuous routine work of each forestry enterprise, and there is little difference in these indicators. As for the specific index, non-commercial forest, environmental label product certification and forest land have the highest weight, accounting for 17.78, 14.76, and 12.50%, respectively. Environmental penalties and environmental certification have the lowest weight, accounting for 2.55, 7.36%, respectively. This shows that forestry enterprises' ecological environmental responsibility has shifted from traditional environmental protection to ecological construction.

Finally, the final combined value can be obtained in Table 3. Qingshan Paper, Sun Paper, and Yong'an Forestry perform best in CEER. Those three companies rank top 3 with scores of 0.498, 0.462, and 0.452, respectively. The result is consistent with the reality of the enterprise and the previous study (Yao and Yu, 2016; Du, 2020). Qingshan Paper, Sun Paper, and Yong'an Forestry have made many efforts in ecological environmental protection and won many honors. Various measures of forest protection and environmental innovation are shown in their annual reports, and they persist in carrying out forest certification and disclosing environmental reports. However, forestry enterprises performed poorly in CEER generally. Qingshan Paper, which ranks first in CEER, had an average score of less than 0.5 in the past 12 years. The mean CEER score of all samples was only 0.274, indicating that there is still much room for improvement in ecological environmental responsibility of forestry enterprises.

6. Discussion

6.1. Time trend analysis of forestry CEER

Figure 1 shows the vertical change in the CEER performance of forestry enterprises from 2009 to 2021. Although the average score of CEER was a bit low, it still shows an apparent upward trend of forestry CEER from 2009 to 2020, which increased from 0.19 in 2009 to 0.42 in 2020. The reason is the increase of forestry resources owned by forestry companies and the improvement of the forestry certification ratio of the listed companies. However, there is a slight decrease in 2021. The minor decrease was mainly due to the decline in environmental investment of some enterprises, which may be related to the COVID-19 pandemic, causing more cautious investment.

The results coincide with Cheng's study, which shows that the forestry environmental responsibility is dynamic; that is, with the change of ecological environment, the environmental responsibility of forestry enterprises will change accordingly (Cheng and Xu, 2018). Vidal and Kozak also found that the environmental concerns of forestry have been moving away from purely environmental issues such as pollution and recycling to the role forestry plays in the global climate (Vidal and Kozak, 2008). Generally speaking, forestry CEER shows an obvious upward trend yearly, which indicates that the forestry enterprises' awareness of forest and ecological environmental protection is

10.3389/fevo.2022.1088804

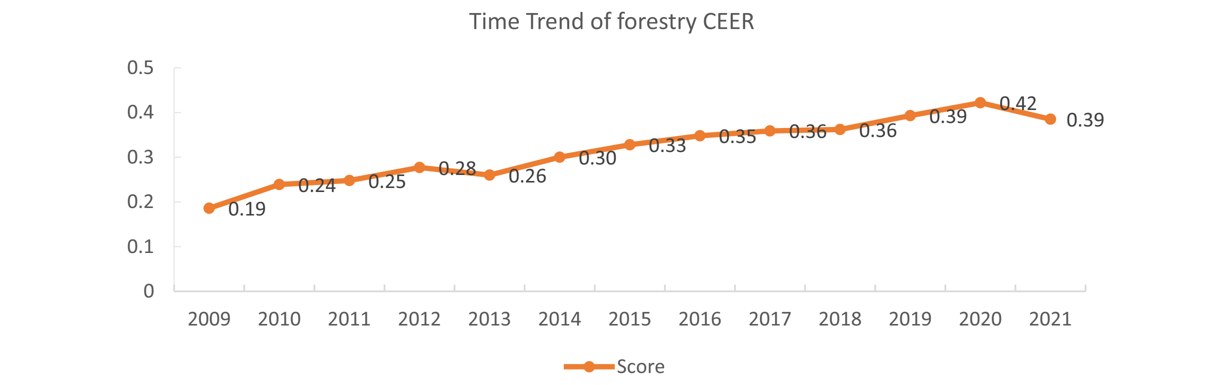

FIGURE 1
Time trend of forestry CEER.

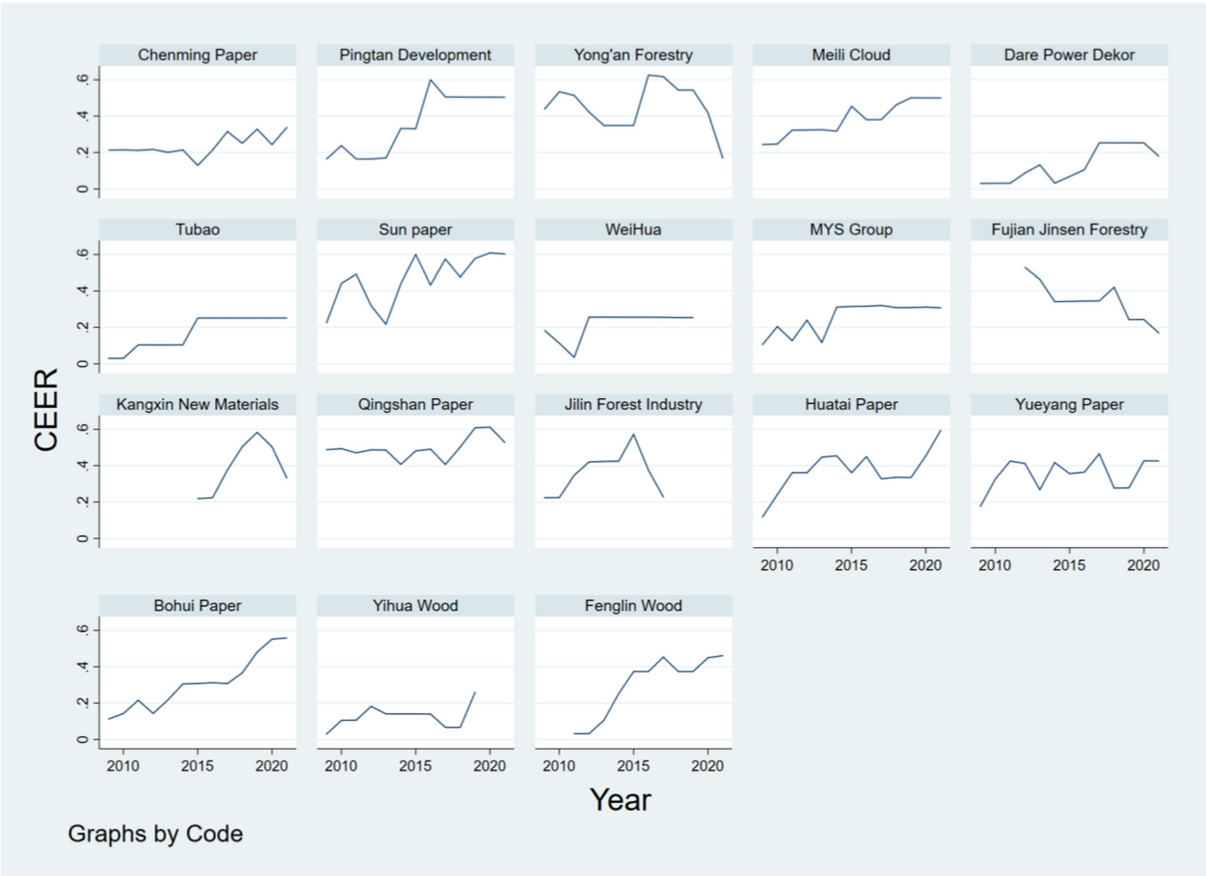

FIGURE 2
Forestry CEER time trend of each company.

constantly improving and also indicates a constantly increasing attention in the country and society toward the ecological environment.

Figure 2 shows the time trend of each company. Most enterprises show an apparent upward trend, while Fujian Jinsen forestry shows a downward trend; Chenming Paper, Qingshan Paper, and MYS Group show a relatively stable trend, while Yong'an Forestry and Sun Paper show relatively intense volatility. It can be seen that forestry CEER performance varies among enterprises.

The reason for the difference is that CEER depends on company characteristic, corporate governance, ownership, forest resources, and other factors. Assisted by green subsidies, firms show better environmental performance than those without (Lin et al., 2015). Jiang et al. (2022) found a positive relationship between environmental protection subsidies and enterprise green innovation. Regulatory policies pressure firms to comply with environmental protection requirements and have a higher quality of environmental performance (Liu et al., 2021). Xie et al. (2020) found that women on boards contribute to environmental and sustainable development strategies. Wang's empirical results showed that stakeholder pressures could significantly affect corporate environmental strategy in developed countries (Wang L. et al., 2020). All those factors may vary considerably among different forestry enterprises, which causes the varied performance of forestry CEER.

6.2. Industry analysis of forestry CEER

Table 8 shows the CEER scores of the three industries in different dimensions. Forest enterprises scored the highest of the total score of CEER, with an average score of 0.350. In terms of specific responsibilities, forest enterprises perform better in ecological construction and green production than other industries. That is because forest enterprises directly take forests as their business objects and carry out abundant forest cultivation work daily. The total score of CEER was followed by forest-board integrated enterprises, with an average score of 0.325, whose responsibility for forest protection is more important than that of other industries, which is related to its high dependence on timber resources. Forest-paper integrated enterprises scored at the bottom of the CEER score, with an average score of 0.301. The CEER of forest-paper integrated enterprises has obvious duality. On the one hand, it is the positive environmental externality brought by forest protection and cultivation. On the other hand, it is the negative environmental externality brought by heavily polluting industries. Therefore, the forest-paper integrated companies put more energy into environmental governance and more effort to establish a green image of the enterprise. Compared with other industries, forest-paper integrated companies focus more on the dimension of environmental input and environmental management.

The Kruskal-Wallis test was further adopted to examine differences between the three groups. It was found that there were no significant differences among different industries in the total score of CEER. However, through the comparison of specific responsibilities, it is found that the dimensions of environmental input, social relations, environmental management, forest protection, and ecological construction were all significant at a 1% level, indicating there is a significant industry heterogeneity in the implementation of forestry ecological environmental responsibilities.

The industry heterogeneity of forestry has been tested by previous studies. Many insights into CSR behavior emerge from this industry-specific analysis. Godfrey pointed out that CSR vary by industry. Manufacturing firms, service firms and banks engaged in different responsibilities; thus, sectors will exhibit different patterns of CSR (Godfrey et al., 2010). In Li's research, the overall CSR of forestry shows no significant difference among industries. However, the regression analysis confirmed a meaningful relationship between the industry and environmental responsibility, which shows that forestry environmental responsibility highly depends on the industry (Li and Gao, 2019). The research of this paper supported and extended the existing results. Different forestry industries attach importance to certain aspects of CEER, and the specific responsibilities of CEER are statistically different.

7. Conclusion

7.1. Conclusion and suggestions

This paper analyzed the content of forestry ecological environmental responsibilities and then evaluated the level of forestry CEER by using a combined evaluation method. Our conclusions can be drawn as follows:

Firstly, when evaluating forestry CEER, results of different single methods are obviously different due to the various data used. The combined evaluation method can obtain more reasonable results because it comprehensively uses all aspects of information and the advantages of every single method.

TABLE 8 Industry comparison of forestry CEER.

Dimension	Forest ($n = 23$)	Forest -broad ($n = 66$)	Forest-paper ($n = 126$)	H value of Kruskal-Wallis	p
Environmental input	0.015	0.034	0.044	10.005	0.007***
Social relations	0.106	0.072	0.075	15.938	0.000***
Environmental management	0.038	0.049	0.06	11.402	0.003***
Green production	0.122	0.094	0.091	1.081	0.582
Forest protection	0.015	0.031	0.012	19.109	0.000***
Ecological improvement	0.054	0.046	0.018	10.361	0.006***
Total score	0.350	0.325	0.301	3.550	0.169

* $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$.

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Second, the particularity of ecological environmental responsibility of forestry enterprises originates from the duality of its impact on the environment: The negative externalities caused by operating and the positive externalities brought by forest resources. Therefore, forestry enterprises bear the dual responsibility of pollution prevention and ecological construction, which should be called "corporate ecological environmental responsibility (CEER)." Regarding specific responsibilities, the most important are ecological construction and green production, and the less important are environmental management and environmental input. The result enriches the definition of corporate environmental responsibility based on the perspective of green resource-based enterprises.

Third, the best CEER companies are Qingshan Paper, Sun Paper, and Yong'an Forestry, while the relatively poor CEER companies are Tubao, Dare Power Dekor, and Yihua Wood. The CEER of forestry enterprises remains poor but shows an apparent upward trend, which indicates an increased awareness of forest and ecological environmental protection of forestry, and the attention of the country and society to the ecological environment.

At last, there is obvious industry heterogeneity in ecological environmental responsibility. Forest enterprises prioritize forest cultivation and pay more attention to ecological construction and green production. Forest-board integrated enterprises depend more on wood so as to focus on sustainable forest development. Forest-paper integrated enterprises pay attention to environmental input and environmental management due to their features of heavy pollution industry. The result brings more attention to the industry characteristic of corporate environmental responsibility.

The above conclusions can be useful for further promoting the ecological benefits of forest companies and developing relevant policies:

First, the government must set up a clear procedure for the identification and evaluation of CEER, which can measure the forestry CEER scientifically and objectively. A unified evaluation procedure makes the CEER of different forestry enterprises comparable, so as to better help society understand the CEER level of various enterprises.

Second, develop a supervisory mechanism for forestry CEER involving the participation of all stakeholders. If the voluntary principle is always adopted, the CEER of forestry enterprises will remain low. The government should give full play to the power of all sectors, such as the media and the public, and formulate corresponding supervision and incentive measures to promote improving the CEER level of forestry enterprises.

Finally, forestry companies should enhance their awareness of CEER and undertake their responsibilities based on their industry characteristics. Forestry enterprises should incorporate CEER into their daily management and strategic objectives and actively participate in China's ecological environment construction.

7.2. Limitations

In this research, there are two limitations. First, CEER covers a wide range of contents. However, due to data availability, the indicators selected in this paper may not cover the entire content of CEER. Typical CEER activities, including forest disaster control, wildlife protection, environmental protection training, and public service activities such as "Earth Hour" were not included in the study, which may result in biased research results. In-depth research is needed to obtain primary data to make the research results more objective. Second, in addition to listed companies, many non-listed forestry enterprises in China also have relatively rich forest resources and can produce ecological benefits. However, these companies were excluded from this study, and future research should involve more samples.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

FL: writing, data collection, and review. WS: data analysis, improve, concept, and English corrections. XL: review, methods, and data analysis. RU: review, editing, discussion, and implications. QC: writing draft, conclusion, revision, and discussion. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Effects of nitrogen addition and seasonal change on arbuscular mycorrhizal fungi community diversity in a poplar plantation

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Arbuscular mycorrhizal (AM) fungi play a crucial role in carbon (C), nitrogen (N), and phosphorous (P) biogeochemical cycling. Therefore, it is essential to determine the seasonal responses of the AM fungal community to N addition to understanding better the ecological processes against a background of intensified N deposition. Based on an ongoing field simulation experiment with five N addition levels (0, 5, 10, 15, and 30 $gN \cdot m^{-2} \cdot a^{-1}$) in a 5-year-old poplar plantation at Dongtai Forest Farm in Yancheng, Jiangsu province, eastern China, soil physicochemical properties, the root colonization rate, and the rhizosphere soil AM fungal community diversity and composition in four seasons (summer, autumn, winter, and spring) were investigated. Meanwhile, the relationships between the characteristics of the AM fungal community and soil environmental factors were analyzed. High-throughput sequencing showed that the dominant genera in the poplar plantation were *Glomus* (average relative abundance 87.52%), *Diversispora* (9.62%), and *Acaulospora* (1.85%). The addition of N significantly increased the root colonization rate in spring. The diversity of the AM fungal community (Chao and Shannon indexes) was primarily affected by seasonal change rather than N addition, and the diversity in summer was significantly lower than in the other three seasons. Redundancy analysis showed that soil temperature, available P, total P, and pH significantly affected the structure of the AM fungal community. It can be concluded N addition primarily influenced the root colonization rate, whereas seasonal change had a notable effect on the AM fungal community diversity. Although seasonal change and N addition greatly influenced the composition, seasonal change exerted a more substantial effect than N addition. These results will improve our understanding of the underground ecological processes in poplar plantation ecosystems.

KEYWORDS

nitrogen deposition, arbuscular mycorrhizal fungi, composition and diversity, season, plantation, eastern China

1. Introduction

Global nitrogen (N) deposition caused by anthropogenic activities is increasing dramatically (Penuelas et al., 2013). The atmospheric N deposition rates have exceeded 20 kg N·ha^{-1}·a^{-1} in the forest ecosystems of central and eastern China (Liu et al., 2011). Intensive deposition of reactive N can induce soil nutrient imbalances, which can alter plant productivity, abundance, and ecosystem function (Bobbink et al., 2010), as well as the soil microbial community diversity and composition in forest ecosystems (Tian et al., 2017; Wang et al., 2018).

Arbuscular mycorrhizal (AM) fungi can form a symbiosis with most terrestrial plants, thereby improving nutrient absorption and stress tolerance. In return, host plants provide photosynthetic products for the growth of AM fungi (Smith and Read, 2008). Consequently, AM fungal associations link the below- and above-ground communities in ecosystems and play crucial roles in carbon (C), N, and phosphorous (P) biogeochemical cycling. The high N availability and soil acidification caused by N deposition can influence AM fungal communities, thereby altering the related soil ecological processes (Lilleskov et al., 2019). Despite this recognition, the effect of N deposition on AM fungal communities remains ambiguous and is the subject of intense scientific debate because previous studies have reported negative (Chen et al., 2017; Williams et al., 2017; Treseder et al., 2018), positive results (Egerton-Warburton et al., 2007), or no effects (Mueller and Bohannan, 2015; Maitra et al., 2021). These discrepancies could have been caused by differences in host plant and ecosystem types, soil nutrient contents (N and P), and N addition amounts and durations (Han et al., 2020). Therefore, it is critical to conduct more studies on this topic better to understand AM fungi's roles in ecosystem function.

AM fungi belong to the new Glomeromycota phylum; there is one class, four orders, 11 families, 25 genera, and about 300 species in this phylum (Redecker et al., 2013). Different AM fungi have contrasting growth strategies, hyphae production, and nutrient absorption abilities (Chagnon et al., 2013). For example, species within the Gigaporaceae family tend to allocate more biomass to external hyphae than intraradical structures, whereas species of Glomeraceae display the opposite tendency (Maherali and Klironomos, 2007). More external hyphae mean more photosynthetic products are needed to support the growth of AM fungi and their hyphal networks. Thus, the AM fungal communities tend to shift from Gigasporaceae to Glomeraceae upon anthropogenic N enrichment and experimental fertilization (Treseder et al., 2018). Furthermore, the soil N/P ratio or P availability also affects the response of AM fungi to N addition. The high production of external hyphae always represents competitive soil exploration and high P solubilization. Soil P limitation stimulated by N addition would increase the abundance of Gigasporaceae members (Egerton-Warburton et al., 2007). These results indicated that different groups of AM fungi might have contrasting responses to N addition in various environments.

Seasonal change can influence AM fungi by modifying climatic factors and the C supply derived from host plants (Dumbrell et al., 2011; Maitra et al., 2021). Seasonal variations of the AM fungal community have been studied in arable, wetland, grassland, and forest habitats. Generally, the density of spores, external hyphal length, and root colonization rates were higher in summer, or the growing period, than in winter and early spring (Mandyam and Jumpponen, 2008). Meanwhile, other studies suggested that AM fungi were not affected by season (Santos-Gonzalez et al., 2007; Maitra et al., 2021). Traditionally, the temporal dynamics of AM fungal community have been described based on the morphological analysis of their characteristic structures of AM fungi. However, some AM fungal structures cannot be precisely identified through their morphologies. Therefore, the resulting compositions will likely differ from field AM fungal communities (Davison et al., 2012). By contrast, molecular techniques can overcome the limitations of the traditional method. Therefore, DNA-based and morphological techniques can be used in ecological studies of the AM fungal community (Vieira et al., 2018).

As a critical tree species for afforestation, poplar has been widely planted in China, especially in the eastern coastal area (Fang, 2008). This region suffers severe N deposition (Liu et al., 2011). Based on a long-term experiment with five N addition levels (0, 5, 10, 15, and 30 gN·m^{-2}·a^{-1}) in a poplar plantation in Dongtai Forest Farm, Jiangsu Province, researchers found changes in soil organic carbon, soil fauna, and the microbial community structure (Bian et al., 2019; Yu et al., 2021). However, the response of AM fungi to N addition in the poplar plantation remains unknown. This study sampled poplar roots and rhizosphere soils in four seasons (summer, autumn, winter, and spring) after 6 years of continuous N additions. The effects of N addition and seasonal change on AM fungi were examined by traditional root colonization identification and high-throughput sequencing. We tested two hypotheses: (1) N addition and seasonal change could alter the AM fungal colonization rate and AM fungal community diversity and composition, and (2) N addition and seasonal change-induced variations of soil physicochemical properties contribute to the variation in the AM fungal community.

2. Materials and methods

2.1. Site description and soil sampling

The study site is located at Dongtai Forest Farm in Yancheng, Jiangsu province, eastern China (E120° 49′, N32° 52′). The region experiences a subtropical northern monsoon climate. The mean annual precipitation is 1,050 mm, and the mean annual temperature is 14.6°C. The growing season extends from May to October, whereas the non-growth season is from November to April. The soil is classified as fluvisol, according to the Food and Agriculture Organization (FAO) of the United Nations (Ge et al., 2018).

The farm was reclaimed from the coastal wetlands by constructing coastal levees. Poplar is one of the main tree species planted on the farm, and the area of the poplar plantation covers approximately 2000 hm^2. The N addition experiment started in May 2012 and involved five N levels in 5-year-old poplar plantations (*Populus deltoides* L. '35'). We selected three sites (25 × 190 m), with the distance between any two sites being >1 km. Each site was divided into five plots (25 × 30 m) with a 10 m wide buffer zone. Five N addition treatments were randomly applied to the plots: N_0 (control), N_1 (5 gN•m^{-2}•a^{-1}), N_2 (10 gN•m^{-2}•a^{-1}), N_3 (15 gN•m^{-2}•a^{-1}), and N_4 (30 gN•m^{-2}•a^{-1}). Nitrogen in the form of NH_4NO_3 has been applied annually during the growing season. Fertilizer with different N concentrations was sprayed into the corresponding plots below the canopy, and the control plots received the same amount of deionized water.

Root and soil samples were collected in July 2018, October 2018, January 2019, and April 2019 (representing summer, autumn, winter, and spring, respectively). Fine roots (< 2 mm) and rhizosphere soils of five poplars with the same growth rate were randomly sampled from each plot. Five samples from the same plot were mixed homogeneously into one sample, resulting in 60 sub-samples. The rhizosphere soils were sieved with a 2 mm mesh and subdivided into two parts. The first part was air-dried for soil physicochemical properties analyses. The other part was stored at −80°C for DNA extraction. The fine roots were washed and cut into 1 cm fragments to determine the proportion of the root colonized by AM fungi.

2.2. Root colonization rate and soil physicochemical properties determination

The root colonization rate was measured using the grid-crossing method (McGonigle et al., 1990). The average soil moisture and temperature of the sampling month were measured using an automatic sensor. A glass electrode determined soil pH in a 1:5 soil/water (w/v) suspension. Soil total nitrogen (TN) and total carbon (TC) were measured using an elemental analyzer (2,400 II, Perkin Elmer, Waltham, MA, United States). Soil-available phosphorus (AP) was extracted with 0.5 M $NaHCO_3$, and total phosphorus (TP) was determined after digestion by alkali fusion. AP and TP were quantified using the Mo-Sb colorimetric method. Ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were quantified using indophenol blue colorimetry and ultraviolet spectrophotometry, respectively (Lu, 2000).

2.3. DNA extraction, amplification, and sequencing

Total DNA was extracted from 0.5 g of frozen soil using a FastDNA kit (MP, Santa Ana, CA, United States). AM fungal 18S rDNA for Miseq pyrosequencing was amplified using a two-step PCR. In the first PCR, the primer pair AML1 and AML2 were used (Lee et al., 2008). The obtained products were used as templates for the second PCR using primers AMV4-5NF and AMDGR (Van Geel et al., 2014). PCR reactions were performed in a 20 μl reaction mixture containing 4 μl of 5× FastPfu buffer, 0.8 μl of each primer, 2 μl of dNTPs, 0.4 μl of DNA polymerase, 0.2 μl of bovine serum albumin (BSA), and 10 ng of DNA template. The PCR condition consisted of an initial denaturation at 95°C for 3 min, 30 cycles of denaturation (95°C for 30 s, 55°C for 30 s, and 72°C for 45 s), and a final extension at 72°C for 10 min. The PCR products were purified using an Axy Prep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, United States) and then quantified using a Quanti Fluor™-ST (Promega, San Luis Obispo, CA, USA). The purified PCR products were sequenced on a Miseq PE250 pyrosequencer (Illumina, San Diego, CA, United States) at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China).

2.4. Bioinformatics and statistical analysis

Raw sequence data were quality-filtered and spliced using FASTP (version 0.19.6[1]) and FLASH (version 1.2.11[2]) software, respectively. Briefly, reads with a quality score < 20, ambiguous nucleotides, reads shorter than 200 bp (excluding barcode and primer sequences), or lacking a complete barcode were removed and excluded from further analysis. The remaining sequences were then clustered into operational taxonomic units (OTUs) at 97% similarity using UPARSE (version 7.1[3]). The taxonomic identification of OTUs was performed by consulting the MaarjAM database (version 81[4]). The Chao and Shannon indexes were calculated after normalizing the read number to 10,561 reads (the minimum sequence number of all samples) in Mothur (Schloss et al., 2009), and the AM fungal community composition was assessed at the genus and species levels. The Bray-Curtis distances of community compositions (based on the OTU compositions) under five N additions and four seasons were calculated using nonmetric multidimensional scaling (NMDS), and the significance of community differences was assessed by analysis of similarities (ANOSIM) in the vegan package in R 3.6 (Dixon, 2003). Additionally, the relationships between the AM fungal community composition and soil physicochemical properties were evaluated using redundancy analysis (RDA). Only the environmental factors with a variance inflation factor (VIF) of less than 10 were retained in the RDA analysis. The significance of the correlation between environmental factors and the community composition was determined using a Monte Carlo test in R (Dixon, 2003). To identify the main and interactive effects of seasonal changes and N addition treatments on the root

1 https://github.com/OpenGene/fastp
2 https://ccb.jhu.edu/software/FLASH/index.shtml
3 http://drive5.com/uparse/
4 https://www.maarjam.botany.ut.ee/

colonization rate and the AM fungal community diversity and composition, multivariate analysis of variance (MANOVA) was performed in SPSS 18.0 for Windows (IBM Corp., Armonk, NY, United States). Lastly, associations between AM fungal community characteristics (including the root colonization rate, diversity, and the relative abundance of AM fungal groups) and environmental factors were determined using Spearman.

3. Results

3.1. Root AM fungal colonization rate

N addition significantly affected the root colonization rate; however, the effect of seasonal change and their interaction was not significant (Figure 1). Generally, with the increase in the level of N addition, the root colonization rate showed an increasing trend. In spring, the root colonization rate under N_0 treatment was significantly lower than that under N addition ($p < 0.05$).

3.2. Arbuscular mycorrhizal fungal community diversity

A total of 1,264,310 high-quality sequences were identified from all 60 samples (ranging from 10,561 to 24,418 reads per sample; mean 21,068), clustered into 227 OTUs. Rarefaction curves suggested that the sequencing depth in this study was sufficient to reflect the community structure in the samples (Supplementary Figure 1). Seasonal changes significantly affected the Chao and Shannon indexes of the AM fungal community ($p < 0.01$, Table 1); however, the effect of N addition and their interaction was insignificant. Compared with summer, the Chao and Shannon indexes of AM fungal community were significantly higher in winter, spring, and autumn.

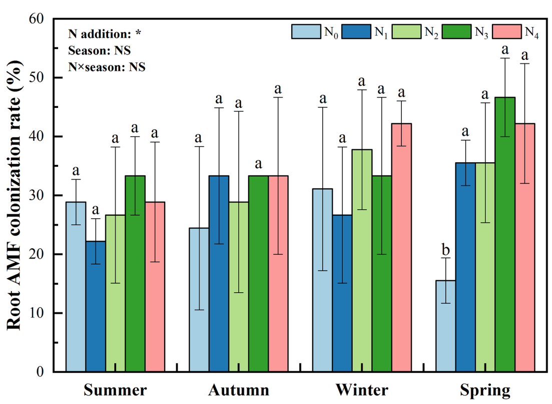

FIGURE 1
Seasonal change in poplar roots' arbuscular mycorrhizal (AM) fungal colonization rate with different nitrogen additions. N_0, N_1, N_2, N_3, and N_4 were 0, 5, 10, 15, and 30 $gN{\cdot}m^{-2}{\cdot}a^{-1}$, respectively. The values are the means of three replicates ± SD. Different lowercase letters within the same season indicate significant differences among nitrogen additions at the 0.05 level. Two-way ANOVA outputs are shown in the figure. NS, not significant; *, $p<0.05$.

3.3. Arbuscular mycorrhizal fungal community composition

Analysis of Similarity (ANOSIM) revealed that the structures of the AM fungal community were significantly influenced by seasonal changes ($R = 0.554$, $p = 0.001$; Figure 2) and N treatments ($R = 0.05$, $p = 0.031$). ANOSIM analysis was also performed on AM fungal community structures among different N additions in the same season. We found that there were significant differences in AM fungal community structures between N additions in spring ($R = 0.772$, $p = 0.001$), winter ($R = 0.704$, $p = 0.001$), and summer ($R = 0.387$, $p = 0.009$), but no in autumn ($R = 0.039$, $p = 0.358$).

The AM fungal community in the poplar plantations was assigned to 4 orders, 7 families, 8 genera, and 43 species. Figure 3 and Supplementary Figure 2 present the AM fungal community compositions on the genus and species level, respectively. The genera *Glomus* (average relative abundance, 87.52%), *Diversispora* (9.62%), *Acaulospora* (1.85%), unclassified Glomeromycetes (0.61%), *Scutellospora* (0.22%), *Archaeospora* (0.12%), unclassified Diversisporaceae (0.05%), and *Paraglomus* (0.005%) were detected in the samples. The genus *Glomus* showed the highest abundance, consisting of 28 species. The genus *Diversispora* could be further divided into three main species: *Diversispora* VTX00060, *Diversispora* VTX00040, and unclassified *Diversispora* (Supplementary Figure 2).

The relative abundances of *Glomus*, *Diversispora*, *Acaulospora*, and *Paraglomus* were significantly affected by N addition treatments and seasonal changes. The abundances of *Glomus* in winter (98.3%) and autumn (93.3%) were higher than those in spring (78.8%) and summer (79.7%). In comparison, the abundances of *Diversispora*, *Acaulospora*, and *Paraglomus* in winter and autumn were lower than those in spring and summer ($p < 0.05$, Figure 3). Compared with N_0 treatment, N addition significantly decreased the relative abundances of *Glomus* and had a trend to increase the relative abundances of *Acaulospora* and *Diversispora*. The relative abundances of *Diversispora* and *Paraglomus* under N_2 treatment were higher than those under the other treatments, while the highest relative abundances of *Glomus* (92.6%) and *Acaulospora* (5.39%) were under N_0 and N_1 treatments, respectively.

3.4. Drivers of the root colonization rate and the AM fungal community diversity and composition

Soil pH, total P, C/N ratio, NO_3^--N, and NH_4^+-N were significantly affected by seasonal change, N additions, and their interaction effects (Table 2). Soil total C, available P, moisture, and

TABLE 1 Seasonal changes in the Chao and Shannon indexes of the AM fungal community in the poplar plantations with different nitrogen additions.

Treatment	Chao				Shannon			
	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring
N_0	57.17 ± 6.92a	76.44 ± 6.71a	61.17 ± 11.97a	75.90 ± 13.13a	1.84 ± 0.51a	2.70 ± 0.31a	2.76 ± 0.10a	3.09 ± 0.14a
N_1	52.56 ± 13.80a	73.72 ± 6.71a	55.58 ± 8.84a	62.61 ± 10.07a	1.97 ± 0.80a	2.76 ± 0.53a	2.72 ± 0.08a	2.80 ± 0.19b
N_2	51.21 ± 1.49a	72.75 ± 6.47a	61.44 ± 6.87a	73.87 ± 3.38a	2.64 ± 0.12a	2.87 ± 0.34a	2.81 ± 0.11a	2.89 ± 0.03ab
N_3	47.17 ± 8.14ab	78.44 ± 3.86a	61.50 ± 8.85a	71.64 ± 10.43a	1.75 ± 0.63a	2.59 ± 0.89a	2.85 ± 0.07a	3.11 ± 0.08a
N_4	34.03 ± 4.65b	75.17 ± 22.25a	60.00 ± 13.81a	75.30 ± 15.75a	1.63 ± 0.60a	2.58 ± 0.88a	2.88 ± 0.19a	3.00 ± 0.17ab
Significance caused by:								
N addition	NS				NS			
Season	**				**			
N × season	NS				NS			

N_0, N_1, N_2, N_3, and N_4 were 0, 5, 10, 15, and 30 $gN{\cdot}m^{-2}{\cdot}a^{-1}$, respectively. The values are the means of three replicates ± SD. Different lowercase letters within the same column indicate significant differences among nitrogen addition treatments at the 0.05 level. Two-way ANOVA outputs are shown in the table: NS, not significant; **, $p < 0.01$.

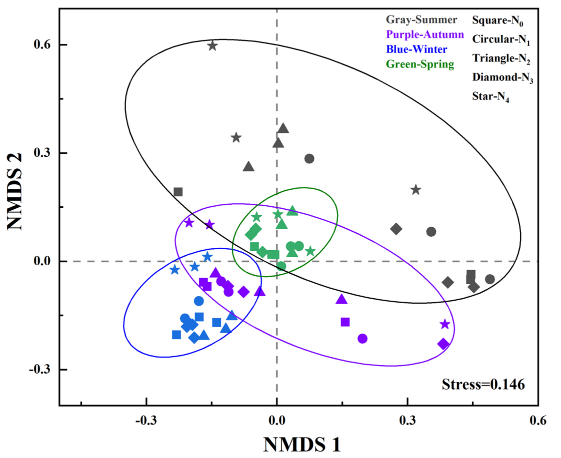

FIGURE 2
Nonmetric multidimensional scaling (NMDS) of seasonal changes in the soil AM fungal community compositions (based on Bray-Curtis distances and the presence of OTUs) in the poplar plantations under different nitrogen additions. N_0, N_1, N_2, N_3, and N_4 were 0, 5, 10, 15, and 30 $gN{\cdot}m^{-2}{\cdot}a^{-1}$, respectively.

temperature were significantly influenced by seasonal change. Specifically, NO_3^--N concentrations increased dramatically from N_0 (6.37 $mg{\cdot}kg^{-1}$) to N_4 (19.42 $mg{\cdot}kg^{-1}$) treatment ($p < 0.01$). Soil temperature decreased from summer (July 2018, 20.23°C) to winter (January 2019, 5.55°C) and then increased from winter to spring (April 2019, 14.64°C). For soil moisture, the order was winter > spring > summer >autumn.

The Chao index was largely associated with NH_4^+-N, available P, pH, NO_3^--N, total P, and total C. The Shannon index was significantly associated with total P, pH, available P, temperature, and total C ($p < 0.05$, Table 3). NH_4^+-N (R = −0.477, $p < 0.01$) and total P ($R = -0.494$, $p < 0.01$) showed the highest correlation with the Chao index and the Shannon index, respectively. The root AM fungal colonization rate was significantly related to the C/N ratio, total C, pH, available P, and NO_3^--N ($p < 0.05$, Table 3).

Nine environmental factors (pH, total C, total P, total N, available P, C/N ratio, NO_3^--N, NH_4^+-N, and soil temperature) were chosen for the RDA analysis, which explained 35.45% (32.89% for the first two axes) of the total variances of the AM fungal community ($p = 0.001$, Figure 4). The Monte Carlo test indicated that the structure of the AM fungal community correlated with soil temperature, available P, total P, and pH ($p < 0.05$, Table 4). The correlation analysis between the relative abundance of the AM fungal genera and environmental factors showed that the relative abundances of *Glomus*, *Diversispora*, and *Acaulospora* correlated significantly with total N, NO_3^--N, and temperature (Supplementary Table 1). The relative abundance of *Scutellospora* correlated positively with available P, total N, and temperature, while *Archaeospora* correlated significantly with total N, moisture, and NH_4^+-N ($p < 0.05$, Supplementary Table 1).

4. Discussion

N addition positively affected the root AM fungal colonization rate in the poplar plantations. Specifically, root colonization rates under N additions were significantly higher than under N_0 treatments in spring (Figure 1). The exchange of P and C is the fundamental function of the plant-AM fungal symbiosis; therefore, the soil P availability can strongly influence the response of AM fungi to N fertilization (Williams et al., 2017). In P-deficient soils, N fertilization-induced P limitation would stimulate plant C allocation to AM fungi to acquire sufficient P. However, in P-rich soil, plant dependency on AM fungi might be reduced, resulting in a decrease in C allocation to AM fungi (Johnson et al., 2013; Han et al., 2020). The soils under the poplar plantation in our study belong to the former category; thus, N addition increased the root AM fungal colonization rate. The correlation analysis also

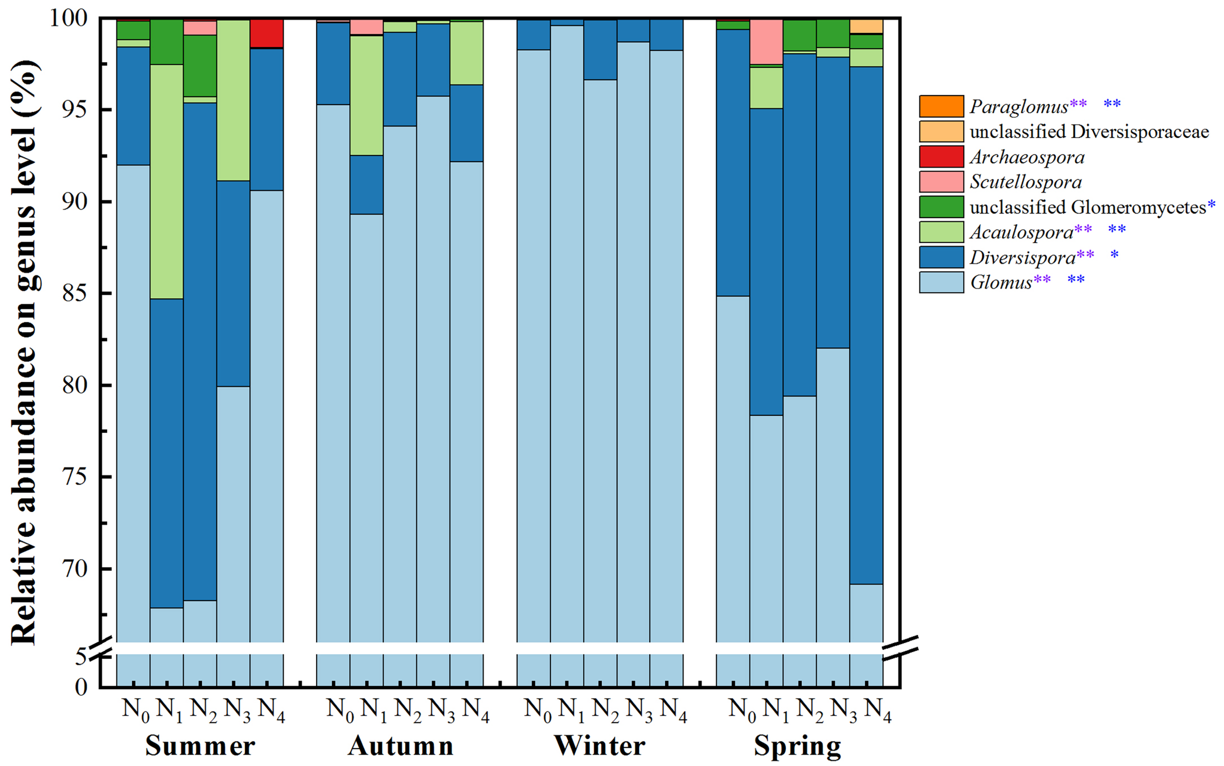

FIGURE 3
Average relative abundances of the AM fungal groups at the genus level in the poplar plantations under different nitrogen additions and seasons. N_0, N_1, N_2, N_3, and N_4 were 0, 5, 10, 15, and 30 gN·m^{-2}·a^{-1}, respectively. The values are the means of three replicates. Two-way ANOVA outputs are shown in the figure: the purple * and ** indicate significant differences among different seasons at the 0.05 and 0.01 levels, respectively, and the blue * and ** indicate significant differences among nitrogen additions at the 0.05 and 0.01 levels, respectively.

showed that the colonization rate correlated negatively with soil available P ($p < 0.05$, Table 3).

Seasonal variation played a notable role in the diversity of the AM fungal community. AM fungi must establish symbiosis with living plant roots to complete their life history; therefore, some studies found strong relationships between the phenology of plants and their mycorrhizal responsiveness. AM fungal characteristics were higher in the growing period than in the dormant season (Mandyam and Jumpponen, 2008). However, in the present study, the Chao and Shannon indexes were significantly higher in autumn, spring, and winter than in summer (Table 1). They tended to be negatively correlated with soil temperature (Table 3). Higher diversity in the colder seasons probably resulted from more even competition for limited C availability among AM fungal species (Dumbrell et al., 2011). In addition, poplar can form associations with AM fungi and ectomycorrhizal (EM) fungi simultaneously (Neville et al., 2002), and increased colonization by EM fungi in summer might influence AM fungal growth.

Although N addition and season influenced the AM fungal community compositions, seasonal change exerted a more substantial effect than N addition (Figure 2). Dumbrell et al. (2011) observed distinct AM fungal compositions in the cooler and warmer seasons. Similarly, the AM fungal assemblages in summer and winter were completely separate in the NMDS plots (Figure 2). The RDA results showed that the AM fungal community composition correlated significantly with temperature (Table 4). The mechanisms of the effect of temperature on the AM fungal communities can be classified as either indirect, by altering soil nutrient availability and host plants' C allocation, or direct, by affecting AM fungal growth (Cotton, 2018). The RDA results also showed that the AM fungal community composition was greatly influenced by available P, total P, and pH (Table 4). N addition and season directly affected soil pH, P, and N availability (Table 2). Consequently, the effects of N addition and season on the AM fungal community composition were partially mediated through the soil (Zheng et al., 2014; Ji et al., 2021). Moreover, previous studies reported an indirect plant-mediated effect of N addition on AM fungal community composition in grassland ecosystems (Liu et al., 2012). In the poplar plantation ecosystem, nine environmental factors chosen for the RDA analysis explained 35.45% of the total variances of AM fungal community. Therefore, other environmental factors that affected the AMF community structure were not considered, e.g., the composition and diversity of the plant community. In future research, their

TABLE 2 Seasonal changes in soil physicochemical properties in the poplar plantations with different nitrogen additions.

Season	Treatment	pH	Total N (%)	Total P ($mg{\cdot}kg^{-1}$)	Total C (%)	C/N ratio	Available P ($mg{\cdot}kg^{-1}$)	NH_4^+-N ($mg{\cdot}kg^{-1}$)	NO_3^--N ($mg{\cdot}kg^{-1}$)	Moisture (%)	Temperature (°C)
Summer	N_0	8.38b	0.20a	871.95ab	1.29b	6.46a	50.22ab	8.53b	8.24c	21.0a	20.23a
	N_1	8.38b	0.17a	911.55a	1.28b	7.55a	57.48a	10.50a	9.94c	20.8a	20.24a
	N_2	8.51a	0.17a	814.07b	1.30b	7.49a	48.54b	7.20c	10.77c	21.2a	20.22a
	N_3	8.50a	0.19a	837.19ab	1.38b	7.35a	53.98ab	9.23b	15.33b	20.9a	20.21a
	N_4	8.36b	0.19a	800.65b	1.68a	9.40a	50.02ab	9.13b	21.41a	21.0a	20.22a
Autumn	N_0	8.52b	0.26ab	815.20a	1.70a	7.54b	26.73ab	4.85ab	3.12c	18.1a	17.33a
	N_1	8.51b	0.66a	789.17a	1.74a	4.86b	21.38b	5.40a	2.87c	18.0a	17.35a
	N_2	8.50b	0.42ab	796.61a	1.36a	4.04b	38.54a	5.28a	11.48b	18.0a	17.31a
	N_3	8.57a	0.04b	785.29a	1.63a	52.15a	19.09b	3.86c	11.07b	18.1a	17.31a
	N_4	8.32c	0.13ab	749.56a	1.56a	11.86b	19.70b	4.18bc	27.53a	17.9a	17.31a
Winter	N_0	8.39c	0.15a	638.34bc	1.55a	10.13b	18.63a	10.51a	12.37c	33.7a	5.52a
	N_1	8.50b	0.14ab	615.03c	1.51a	10.54b	17.16a	8.28bc	12.55c	33.9a	5.53a
	N_2	8.60a	0.16a	682.88a	1.53a	9.63b	23.27a	7.46c	13.90b	33.4a	5.58a
	N_3	8.63a	0.16a	655.06ab	1.70a	10.78b	23.37a	9.87ab	16.34a	34.1a	5.54a
	N_4	8.56ab	0.13b	633.14bc	1.56a	12.31a	20.97a	9.30abc	16.79a	33.6a	5.56a
Spring	N_0	8.63bc	0.14b	599.37ab	1.54d	10.76bc	22.30a	9.49a	1.75b	27.9a	14.63a
	N_1	8.59c	0.21a	609.63a	2.92a	13.85a	21.08a	7.76b	5.28b	27.8a	14.63a
	N_2	8.65b	0.21a	588.53b	2.35b	11.19bc	21.64a	6.16c	10.49a	27.8a	14.63a
	N_3	8.78a	0.19a	599.16ab	2.13bc	11.47b	24.23a	9.26a	11.75a	27.9a	14.62a
	N_4	8.64bc	0.21a	597.26ab	1.97c	9.56c	17.67a	7.31b	11.96a	27.9a	14.66a
Significance caused by:											
N addition		**	NS	*	NS	**	NS	**	**	NS	NS
Season		**	NS	**	**	*	**	**	**	**	**
N × season		**	NS	*	**	**	NS	**	**	NS	NS

N_0, N_1, N_2, N_3, and N_4 were 0, 5, 10, 15, and 30 $gN{\cdot}m^{-2}{\cdot}a^{-1}$, respectively. The values are the means of three replicates. The means followed by different letters in the same column and season are significantly different ($p < 0.05$). Two-way ANOVA outputs are shown in the table. NS, not significant; **, $p < 0.01$; *, $p < 0.05$.

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TABLE 3 Spearman correlation coefficients between the diversity of the AM fungal community and environmental factors (n=60).

Factors	pH	Total N	Total P	Total C	C/N ratio	Available P	NH_4^+-N	NO_3^--N	Moisture	Temperature
Chao index	0.333**	0.123	−0.286*	0.286*	0.177	−0.376**	−0.477**	−0.313*	−0.231	−0.177
Shannon index	0.477**	0.000	−0.494**	0.305*	0.253	−0.466**	−0.175	−0.098	0.091	−0.382**
Colonization rate	0.301*	−0.096	−0.232	0.322*	0.323*	−0.285*	−0.050	0.259*	0.203	−0.237

** and * indicate significant correlations at $p < 0.01$ and $p < 0.05$, respectively.

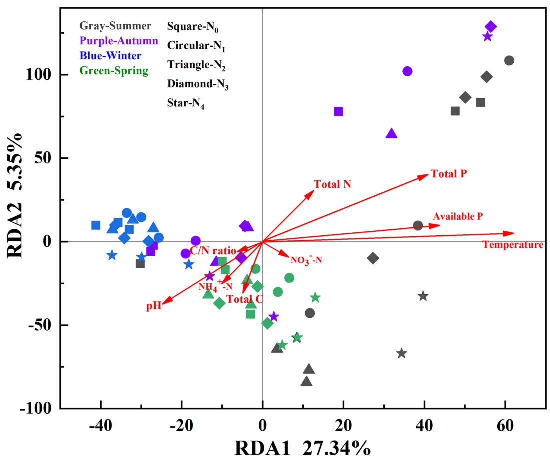

FIGURE 4
RDA for soil AM fungal community compositions (based on the species level) and environmental factors in the poplar plantations under different nitrogen additions in four seasons. N_0, N_1, N_2, N_3, and N_4 were 0, 5, 10, 15, and 30 gN·m^{-2}·a^{-1}, respectively.

impacts on the AM fungal community could be further investigated.

Generally, the AM fungal communities in poplar plantations were assigned to 4 orders, 7 families, 8 genera, and 43 species using high-throughput sequencing. The dominant genera were *Glomus* (average relative abundance 87.52%), *Diversispora* (9.62%), and *Acaulospora* (1.85%) (Figure 3). Using molecular techniques, former studies examined the structure of AM fungal communities in some forest ecosystems. In a subtropical forest, the dominant AM fungal groups across three seasons were Glomeraceae (78.82%), Archaesporaceae (11.06%), Claroideoglomeraceae (3.49%), Gigasporaceae (3.33%), Acaulosporaceae (1.61%), and Diversisporaceae (1.60%) (Maitra et al., 2021). In a rainforest, the AMF community was dominated by *Glomus* (96.72%), *Acaulospora* (2.42%), and *Scutellospora* (0.63%) (Pereira et al., 2022). Similarly, the genera *Glomus*, *Diversispora*, *Scutellospora*, and *Paraglomus* accounted for more than 95% of the sequences in a natural mixed broadleaf-conifer forest (Ji et al., 2021). Therefore, the dominant groups of the AM fungal community in different forest ecosystems vary. The study of the structure of the AM fungal community in the ecosystem of a poplar plantation can provide references for the exploration of their function.

Furthermore, in this study, N addition and season influenced the relative abundances of the dominant AM fungal groups (Figure 3). Compared with N_0 treatment, N addition significantly decreased the relative abundances of *Glomus*, and had a trend toward increasing the relative abundances of *Diversispora* and *Acaulospora*, as demonstrated in previous investigations (Liu et al., 2015; Chen et al., 2017). The AM fungal community structure shifts were related to the competitive dynamics among AM fungi, changes in environmental conditions, or both (Dumbrell et al., 2011). Fertilization has been shown to have significant effects on the AM fungal population. For example, suppose the host plant reduces the allocation of C to the mycorrhiza under N addition. In that case, the intensified competition among AM fungi might result in a dominance of the AMF taxa, which can adapt to environments rich in N and limited in C (Johnson et al., 2015).

Moreover, because different AM fungi have contrasting soil exploration and P solubilization abilities, soil P availability affected the dependence of host plants on mycorrhizae (Treseder et al., 2018). The abundances of *Glomus* in winter and autumn were higher than in spring and summer, while the abundances of *Diversispora* and *Acaulospora* showed an opposite trend. The genus *Glomus* possessed a high sporulation rate and colonization ability *via* spore dispersal or mycelium fragments (Daniell et al., 2001); therefore, they were more likely to survive in relatively severe winter and autumn conditions.

5. Conclusion

In this study, we analyzed the seasonal changes of the root AM fungal colonization rate after six-year N addition in poplar using traditional morphological identification. We also detected the composition and diversity of AM fungal community in the rhizosphere soil using high-throughput sequencing. Our results showed that N addition primarily influenced the root colonization rate. In contrast, seasonal change had a notable effect on the Chao and Shannon indexes

TABLE 4 Relationships between the composition of the arbuscular mycorrhizal (AM) fungal community and environmental factors revealed by Monte Carlo tests (n=60).

	pH	Total N	Total P	Total C	C/N ratio	Available P	NH_4^+-N	NO_3^--N	Temperature
R^2	**0.100**	0.045	**0.242**	0.042	0.004	**0.306**	0.028	0.012	**0.648**
P	**0.050**	0.268	**0.001**	0.318	0.928	**0.001**	0.460	0.700	**0.001**

Significant correlations are marked in bold.

of the AM fungal community. Meanwhile, both the seasonal change and N addition impacted the composition of the AM fungal community. Soil temperature, available P, total P, and pH were the main drivers for the seasonal dynamics of AM fungal community in this poplar plantation.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI; SAMN31857539- SAMN31857598.

Author contributions

SP, ZG, and LM: designed the project and provided the funding. SP and MB: collected the samples and conducted the laboratory analysis. SP, WX, and ZG: performed the bioinformatics and statistical analysis. SP: wrote the original draft of the paper. LM: revised the manuscript and contributed to the conception. All authors have read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.1101698/full#supplementary-material

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Phosphorus extractability in relation to soil properties in different fields of fruit orchards under similar ecological conditions of Pakistan

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Productivity of an orchard generally depends upon the fertility of the soil and the nutrient requirements of the fruit trees. Phosphorus (P) extractability from soils influences the P sorption, release patterns, and P bioavailability. A study was carried out to investigate P extractability *via* seven extraction methods in relation to soil properties in three fruit orchards. In total, 10 soil samples were collected from each fruit orchard, namely, citrus (*Citrus sinensis* L.), loquat (*Eriobotrya japonica* L.), and guava (*Psidium guajava* L.), located in similar ecological conditions to the Haripur district of Pakistan. Available P in the soil was extracted using deionized H_2O, $CaCl_2$, Mehlich 1, Bray 1, Olsen, HCl, and DTPA methods. Selected soil properties [pH, electrical conductivity (EC), soil organic matter (SOM)], texture, cation exchange capacity (CEC), macronutrients, and micronutrients were also determined. Soils sampled from orchards indicated significant differences in soil properties. Orchards have sequestered more amount of C stock in soil than without an orchard. The extractability of P from soils was profoundly affected by P extraction methods. The average amount of extractable P was relatively higher in those soils where the total amount of P was also higher. These methods extracted different pools of soil P with varying P concentrations regulated by the soil properties. Phosphorus amounts extracted were varied in the order of HCl > DTPA > Mehlich 1 > Bray 1 > Olsen > $CaCl_2$ > water. Among orchards, a higher amount of P was found in soils of loquat followed by citrus and guava orchards. Regardless of the method, subsurface soil got a lower concentration of extractable P than surface soil in all orchards. The extractable P was highly associated with soil properties. DTPA extractable P was related to SOM soil clay content and CEC by R^2 values of 0.83, 0.87, and 0.78, respectively. Most of the extraction methods were positively correlated with each other. This study indicated that SOM inputs and turnover associated with orchard trees exhibited a substantial quantity of extractable P in soils. Predicting available P in relation to its bioavailability using these methods in contrasting soils is required.

KEYWORDS

soil quality (SQ), orchard species, nutrient, arid region, phosphorus

1 Introduction

Fertilization of orchards generally depends on the fertility of the soil and the nutrient requirements of the fruit trees. Fruit plants require essential nutrients such as nitrogen (N), phosphorus (P), and potassium (K) for their growth and fruit production. Regular replenishment of nutrient elements is required for the quality of fruit trees. Soil nutrients play a vital role when present in the soil in an adequate quantity for healthy fruit trees. Nitrogen is required for the vigorous vegetative and floral growth of trees (Gul et al., 2006). Phosphorus is important for the normal cell division, growth, and establishment of sugar-phosphate (Zhang et al., 2019). Soil P is an essential nutrient for plant growth and biomass production (Smith et al., 2015). The provision of P is essential for living organisms as it involves major processes of metabolism, e.g., energy transfer in the form of adenosine triphosphate (ATP). During the process of photosynthesis, plants depend on P nutrients to get energy (Ruttenberg, 2003). Therefore, the importance of P for plants is highly recognized. The optimal quantity of macronutrients is essential for quality fruit production (Aggelopoulou et al., 2011). The deficiency of P in soils retards the growth and productivity of fruit trees (Nazarkiewicz and Kaniuczak, 2012). The quality of fruit production is also associated with the availability of P (Li et al., 2021). Phosphorus is essential for the physiological and biochemical processes of plants (Simpson et al., 2011).

The quality of soil is regulated by both physical and chemical properties of soils and their mutual interactions (Carter, 2002). The availability of nutrients from soil depends upon the soil properties in the rhizosphere (Jiang et al., 2009). These interactions are essential for the balance of the agroecosystem (Cataldo et al., 2021). The physical indicators (e.g., aggregate stability, soil texture, bulk density, and soil porosity) and chemical indicators [e.g., soil organic carbon (SOC), electrical conductivity (EC), pH, and cation exchange capacity (CEC)] for the quality of soil have been reported (Zornoza et al., 2015). The productivity of soil depends on several soil physicochemical properties. For instance, soil texture influences soil biophysical properties and is interrelated with soil fertility and soil quality (Upadhyay and Raghubanshi, 2020). The soil pH affects the bioavailability of nutrients (Berbecea et al., 2011). The optimum soil pH controls the microbial activities in the soil and enhances the availability of mineral nutrients (Jarociński, 2005). Plants absorb P in the form of orthophosphate ions $H_2PO_4^-$ and HPO_4^{2-} (Becquer et al., 2014). The uptake of phosphate decreases as the pH of the soil solution increases (White, 2012).

Besides soil properties, orchard production is also controlled by plant nutrients in the soil (Zia et al., 2006). The deficiency of plant nutrients is a major constraint to the productivity and sustainability of soils (Chaudhari et al., 2012). Poor soil fertility leads to low fruit productivity (Kai et al., 2016). Optimal soil fertility with adequate nutrients is required for better yield (Hoying et al., 2004; Brunetto et al., 2015). The concentration of P in the soil is affected by the interaction of soil properties (Hinsinger, 2013; Messiga et al., 2015). The livelihood and nutritional security of farmers depend on fruit production; therefore, the restoration of the soil is important for the orchard sustainability (Tejada et al., 2006). Nutritional deficiencies in horticultural gardens are common (Urade et al., 2019). The soil of the fruit trees needs to be analyzed for enhancing nutrient management. Average yields of fruit crops in Pakistan are lower than potential yields due to the imbalanced proportion of plant nutrients in soil despite the favorable climatic conditions (Zia et al., 2006). Measurement of available P in soil is required for efficient P use. Phosphorus application to soils is generally based on soil analyses, for which different soil P extraction methods have been used (Wuenscher et al., 2015). In less developed soils, the ranges of average total P are 200–800 mg/kg (Nwoke et al., 2003). Phosphorus characterization in soils is important because P forms influence P desorption/release patterns and their bioavailability. This also depends on the precise measurement of the phyto-availability of P from soil. The average amount of organic P has been reported as 30–65% of the total P (Condron and Tiessen, 2005; Bibi et al., 2022). In soil, major forms of P are as follows: (1) dissolved P in soil water, (2) sorbed P of clay/mineral surfaces or Al and Fe oxides, (3) P in the primary phosphate minerals, and (4) P in living organisms and organic substances. Therefore, numerous extraction methods of soil P have been developed depending on the extracting agents and soil properties. Different methods have been reported for the extraction of distinct pools of soil P, and the extractability of a given pool has been influenced by the soil properties to different extents (Zehetner et al., 2018).

Applications of chemical P fertilizers and animal manure to agricultural land improve the soil P fertility (Shen et al., 2011). Phosphorus in soils is supplied from the weathering process of the primary mineral apatite (Zhou et al., 2018) and the application of both inorganic and organic fertilizers. A small portion of total P in soils is available for agricultural plants and microorganisms (Sayers et al., 2008) because most of the P is strongly bound in soil particles and partially weathered material or occluded in secondary minerals. Application of chemical fertilizers and good crop and soil management can optimize P availability. The relationships between extractable P and plant-available P are predicted by keeping in view soil properties and P forms (Delgado and Torrent, 2001). In total, 16 methods of extraction for the available P have been reported (Tóth et al., 2014). Estimating available P in soils is important for sustainable nutrient management. Therefore, investigating P fertility in soils is desirable for the productivity of fruit orchards. Studies on the availability of P from orchard soil in relation to soil properties for better yield potential and quality of fruit in the Haripur district of Pakistan are lacking. The quality of soil is associated with the mutual interactions of the physicochemical and biological properties of soils (Messiga et al., 2015). Soil quality and restoration of plant nutrients in fruit orchards are important for sustainable productivity.

Several P extraction methods have been utilized to extract the inorganic form of P, which is also considered bioavailable. Soil properties apparently influence the extraction of P from soils; therefore, the availability of P from soil differs with an extracting agent (Haque et al., 2013). Moreover, an extraction method needs to be identified, which may accurately reflect the available P in soils of different land uses. The interrelation of P extractability from soils and major soil properties in field conditions of orchards has not been sufficiently reported. The study hypothesized the differences in P extraction from soils of three orchards. Research on the availability of P in relation to soil properties is necessary for better yield potential of orchards. Therefore, the objective of the study was to investigate P extractability using seven extraction methods in relation to soil properties under the field conditions of three fruit orchards. Investigation of available P in an agroecosystem is important to adopt appropriate fertilizer use and soil management. This study may serve as a guideline for the efficient and sustainable management of fruit production. This would provide an opportunity to attain social and economic security for the poorly resourced farmers of the region.

2 Materials and methods

2.1 Soil sampling and analyses

The soil was sampled from three types of fruit orchards, namely, citrus (*Citrus sinensis* L.), loquat (*Eriobotrya japonica* L.), and guava (*Psidium guajava* L.), located in the Haripur district of Hazara Division, Pakistan (**Figure 1**). These orchards were selected because of their abundance in the area. The weather of Haripur has relatively high temperatures and precipitation is evenly distributed throughout the year. The average rainfall is less than 550 mm. The average temperature in summer and winter ranges between 2 and 40°C. Loquat and citrus species are evergreen trees, and guava is a deciduous fruit species. These orchards were 12–15 years of age. In each orchard, 10 random subplots (5 m × 5 m) were selected for soil samples from the two layers of soil (0–25 and 25–50 cm depths) as composite soil samples (Sumera et al., 2022). There were ten soil samples from each orchard collected separately from both soil depths. The experiment was a factorial (3 types of orchards × 2 soil depths), resulting in 6 experimental units arranged into a completely randomized block design. There were three fruit orchards. Thus, the total number of soil samples was 60. The land features have been properly considered during the soil sampling. These soil samples were mixed appropriately in plastic bags. Composite soil samples were taken from the adjoining fields of each orchard as control soil. The control fields have been cultivated with cereal crops (wheat and maize rotation) for a longer period. The topography of the orchard and crop land was flat. Soil samples were collected randomly from each core with an auger and kept in polythene bags under moist conditions. The materials (stones, granules, plant parts, and leaves) were carefully removed from soil samples. Samples were air-dried, ground, and passed *via* a 2-mm sieve. There were three types of orchards and two layers of soil. The adjacent field of the orchard was considered as a control treatment.

Soil samples were analyzed for physical and chemical properties, i.e., pH, EC, organic matter, texture (sand, silt, and clay), potassium (K), magnesium (Mg), calcium (Ca), and sodium (Na). The soil organic matter (SOM) was calculated by the dry combustion method (Cambardella et al., 2001). Total N content was determined using the Kjeldahl method. The pH was determined using a pH meter (Model: HANNA HI 8520) and EC by electrical conductivity meter (Model: 4320 JENWAY) at a ratio of 1:5 of soil and water (Chi and Wang, 2010). Soil texture was determined using a hydrometric method (Huluka and Miller, 2014). Soil bulk density was estimated by a core method (Throop et al., 2012) using the mass of soil (g) in relation to the volume of soil (cm^3). Concentrations of total macro- and micro-elements (P, Ca, Mg, K, Zn, Cu, Mn, and Fe) in soil were determined after digesting the soil samples in (1:3) mixture of perchloric acid ($HClO_4$) and nitric (HNO_3) acid. The contents were determined using an atomic absorption spectrophotometer

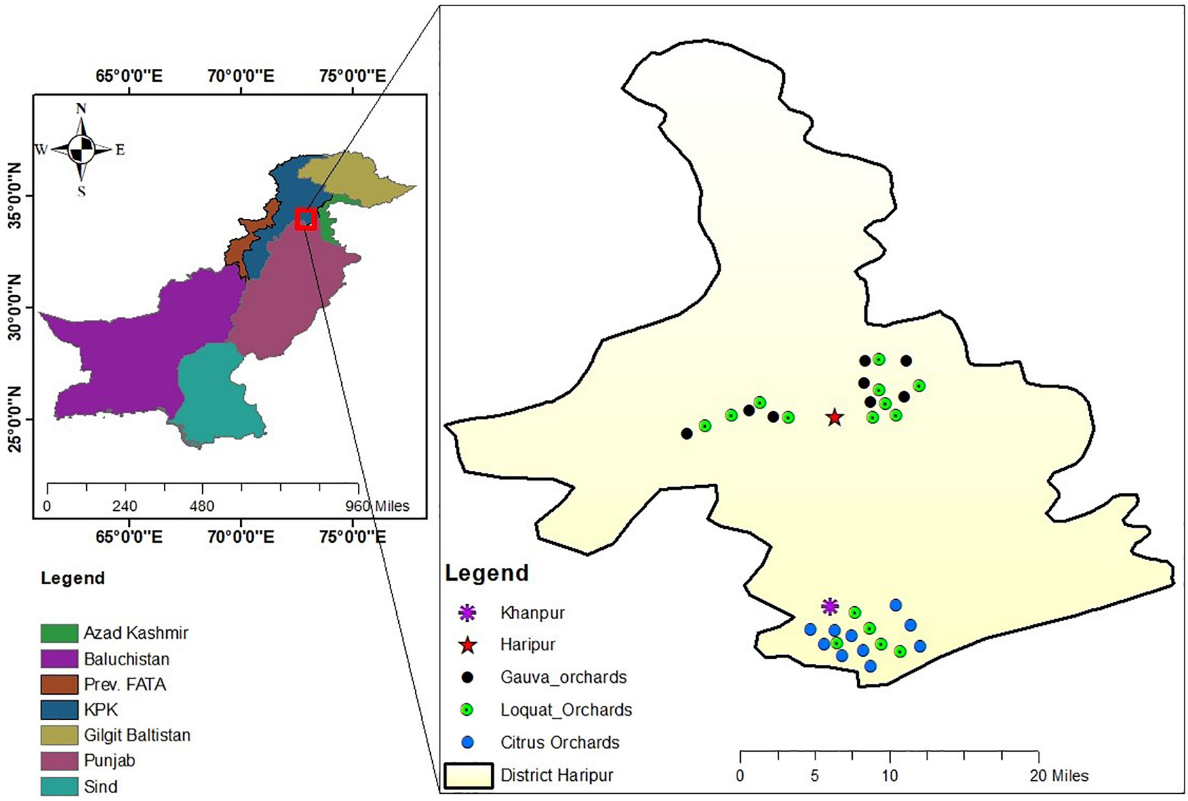

FIGURE 1
Study area of Haripur district, Pakistan.

(AAS) (Model: Analyst 700, Perkin Elmer) (Miller et al., 2013). Calcium, Mg, and K were extracted by ammonium acetate and these exchangeable cations were determined using AAS.

The total carbon stock for the 0- to 30-cm soil layer was estimated based on a hectare.

$$C = \left[BD\ soil * Depth\ soil * C\right] * 100$$

where C = carbon (t ha^{-1}); BD soil = soil bulk density (g cm^{-3}); Depth soil = soil depth.

In this equation, C is expressed as a decimal fraction, e.g., 2.2% carbon is expressed as 0.022 in the equation.

2.2 Phosphorus extraction methods

(1) Available P content from soil was extracted using deionized water. During this method, 10 g of soil was added to 50 ml of deionized water. Samples were shaken for 1 h and centrifuged at 10,000 rpm for 10 min and filtered *via* a 0.22-µm filter paper. The water extraction method determines dissolved or readily soluble P forms in soil (Zehetner and Wenzel, 2000). (2) Calcium chloride ($CaCl_2$): the $CaCl_2$ extraction was prepared following Houba et al. (2000). This is based on the principle of a salt solution (dilute) making some ion exchange process, but it depends on the dissolution of P in extraction solution (Wuenscher et al., 2015). (3) Olsen method: in this method, 0.5 M sodium bicarbonate ($NaHCO_3$) solution (at 8.5 pH) was used for the extraction of P from the soil (Olsen et al., 1954; Otabbong et al., 2009). (4) Mehlich 1 method: for this method, a mixture of 0.05 M hydrochloric acid (HCl) + 0.025 M sulfuric acid (H_2SO_4) was used to dissolve Fe and Al phosphates and adsorbed P (Mehlich, 1953). (5) By Bray and Kurtz P 1 method: P contents were extracted with extracting solution of 0.025 M HCl in 0.03 M NH_4F (Bray and Kurtz, 1945; Sims, 2000). (6) Hydrochloric acid (HCl) method: this method dissolved the inorganic P using 0.5 mol/L HCl (Pagliari and Laboski, 2012). (7) DTPA extractable P method: in this procedure, the P associated with apatite was dissolved and aluminum (Al) and iron (Fe) bound P was extracted (Kuo, 1996; Memon, 2008), using 1 M ammonium bicarbonate (NH_4HCO_3) + 0.005 M diethylene triamine penta acetic acid (DTPA) solution (at pH 7.6) (Tan, 1995). During each procedure, the soil samples were shaken and centrifuged at 10,000 rpm for 10 min, and then, the supernatants were filtered through a 0.22-µm filter paper. The samples were analyzed for P contents using the phosphomolybdate blue color method *via* a spectrophotometer (Model: LI-UV-7000) at 710 nm (Murphy and Riley, 1962).

For the determination of the total concentration of P, the soil sample was digested in a mixture (1:3) of perchloric ($HClO_4$) and nitric (HNO_3) acids. For wet digestion, a soil sample weighing 5 g was transferred into a flask, treated with 24 ml of concentrated HNO_3, and left to digest overnight. After adding 8 ml of $HClO_4$, the flask was heated gently until the digestion of soil material. Upon cooling, contents were filtered through Whatman No. 40 filter paper and the desired volume was diluted with deionized water. The samples were analyzed for P contents using the phosphomolybdate blue color method *via* a spectrophotometer (Model: LI-UV-7000) at 710 nm (Murphy and Riley, 1962).

Data were statistically analyzed using a StatView software (SAS, 1999). The analysis of variance (ANOVA) technique was used to differentiate soil properties among three orchards, and the level of significance was determined *via* the least significant difference (LSD) value at $p < 0.05$. A summary of a two-way analysis of variance for orchard effects on soil properties is also given (**Table 5**). The relationship of extractable P using the above methods with selected soil properties was determined using regression plot analysis.

3 Results

3.1 Description of soil properties

Soil samples collected from different orchards showed significant differences among most of the soil properties ($p < 0.05$) (**Tables 1–3**). The different uses of land with fruit orchards significantly affected the soil properties when compared to the control soil, i.e., without an orchard. The orchard trees brought tangible changes in the chemical composition depending on the nature of the soil and the type of orchard. A higher amount of SOM was observed in orchard soil as compared to control soil irrespective of the fruit orchard. Fallen leaf litter of citrus, guava, and loquat orchards have accumulated 2.6, 1.9, and 3.1 times more SOM in the surface soil (0–25 cm) than in the control fields. In subsurface soil (25–50 cm), the SOM contents were 3.3, 2.4, and 3.9 times higher than the respective control soil of fruit orchards. Orchard soil accumulated a substantial amount of C stock (t ha^{-1}) when compared to the respective control fields (**Figure 2**). Loquat and citrus trees have apparently sequestered more amount of C stock than the guava orchard after comparing with the control soil. This phenomenon could be associated with higher biomass, plant growth pattern, and litter inputs in soil CEC of soil, which was higher in soil planted with loquat trees followed by citrus and guava trees. The bulk density of soil was lower in orchard soil with a higher amount of SOM. The orchard soil exhibited slightly higher pH values than the control soil. The pH of the surface soil was higher than subsurface soil, regardless of the tree species. Under the orchard conditions, SOM, CEC, and essential plant nutrients were found higher in the topsoil as compared to the subsoil.

Nutrient concentrations (N, P, Ca, Mg, and K) were higher in soils under horticultural trees. The concentrations of nutrients were found as loquat > citrus > guava (**Tables 2, 3**). Total P contents were enhanced by citrus, guava, and loquat orchards by 9.3, 18.6, and 53.2%, respectively, in the surface soil as compared to respective control soils. The contents were increased in subsoils under the orchard conditions by 11.1, 13.7, and 29.9%, respectively. Total N contents were enhanced by citrus, guava, and loquat orchards by 24.5, 40.1, and 107.6%, respectively, in the surface soil. The N contents were increased in subsurface soils under these orchards by 45.5, 30.0, and 72.2%, respectively. The cationic elements were differentiated in soils among tree species as K > Ca > Mg > Na. Micronutrients in soils were found in the order of Cu < Zn < Mn < Fe (**Table 3**). Manganese concentration was higher in the soil of the citrus orchard followed by guava and loquat. Copper was higher in the soil after being cultivated with the loquat species and lower in the soil of citrus. Iron concentration was achieved higher in soil under loquat and citrus compared with guava. Zinc was substantially higher in the citrus orchard followed by guava when compared to the soil samples of the loquat orchard.

3.2 Phosphorus extraction methods

The average amount of extractable P was found significantly higher ($p < 0.05$) in those soils of fruit orchards where the total amount of P was higher. The amounts of extractable P *via* different extractants were varied in the order of HCl > DTPA > Mehlich 1 > Bray 1 > Olsen > $CaCl_2$ > water (**Figures 3–7**). Almost all extractants exhibited an identical pattern of P release from soil samples, i.e., higher from orchard soil and lower from control soil. The widest range between the lowest and highest P value was found for Mehlich 1, where the highest P value was 579 mg kg^{-1} and the lowest value was 268 mg kg^{-1} (**Figure 4A**). The narrowest range was observed for the HCl extraction among soils, where the maximum extracted P was 549 mg kg^{-1}, and the minimum value was 425 mg kg^{-1} (**Figure 5B**). Irrespective of the extraction method used, the average amount of extractable P was found higher in soils in the order of loquat > citrus > guava orchard. Water extractable P concentrations in the surface soil were achieved as 223, 183, and 181 mg kg^{-1} in loquat, guava, and citrus orchards, respectively. Adjacent control fields of these orchards exhibited water extractable P in the topsoil (0–25 cm) as 104, 129, and 160 mg kg^{-1}, respectively. The lowest extractable P concentrations in soils were found for the water extraction method, and the highest amounts were extracted by the method used for total P. The P concentrations extracted with $CaCl_2$ in the surface soils were obtained in the control fields of citrus, guava, and loquat as 145, 167, and 164 mg kg^{-1}, respectively.

TABLE 1 Changes in soil properties as affected by three types of fruit orchards.

Sites	Soil depth	pH	EC dS m^{-1}	SOM (%)	$CaCO_3$ (%)	Clay (%)	Texture	CEC meq/100 g	BD g cm^{-3}
CC	00–25	7.8 ± 0.11c	0.24 ± 0.01g	1.8 ± 0.10f	6.4 ± 0.11a	23 ± 1.5f	SCL	16.2 ± 1.2cd	1.12 ± 0.04c
	25–50	7.6 ± 0.02cd	0.25 ± 0.01fg	1.5 ± 0.12h	6.5 ± 0.12a	28 ± 2.2d	SCL	14.3 ± 1.0e	1.23 ± 0.03b
CO	00–25	8.2 ± 0.03b	1.28 ± 0.03c	4.6 ± 0.10ab	6.6 ± 0.09a	34 ± 1.2ab	SC	18.9 ± 1.2c	1.09 ± 0.02d
	25–50	7.9 ± 0.03c	1.29 ± 0.02c	4.8 ± 0.07a	5.9 ± 0.11c	36 ± 1.6a	SC	18.5 ± 1.3c	1.12 ± 0.02c
GC	00–25	8.3 ± 0.07b	0.28 ± 0.01f	1.6 ± 0.06f	5.7 ± 0.08c	16 ± 1.2h	SL	17.0 ± 0.9c	1.21 ± 0.04b
	25–50	7.8 ± 0.04c	0.38 ± 0.02e	1.4 ± 0.02fg	6.2 ± 0.05ab	18 ± 1.2gh	SL	16.5 ± 0.7cd	1.23 ± 0.01b
GO	00–25	8.5 ± 0.09ab	1.29 ± 0.03c	3.0 ± 0.03e	5.8 ± 0.06c	20 ± 2.0g	SL	22.5 ± 0.7b	1.23 ± 0.01b
	25–50	8.4 ± 0.07b	1.23 ± 0.03d	3.3 ± 0.04d	5.1 ± 0.06d	32 ± 1.3c	SCL	14.3 ± 0.4e	1.30 ± 0.03a
LC	00–25	7.8 ± 0.02c	0.27 ± 0.01f	1.6 ± 0.04f	6.5 ± 0.09a	28 ± 1.7d	SCL	14.8 ± 0.3e	1.20 ± 0.03b
	25–50	8.2 ± 0.05b	0.26 ± 0.01f	1.1 ± 0.02i	6.6 ± 0.10a	26 ± 1.4e	SCL	13.3 ± 0.8e	1.11 ± 0.02c
LO	00–25	8.7 ± 0.05a	1.33 ± 0.02b	4.9 ± 0.03a	5.7 ± 0.11c	36 ± 2.1a	SCL	27.6 ± 1.4a	1.06 ± 0.01d
	25–50	8.4 ± 0.04b	1.37 ± 0.04a	4.3 ± 0.03c	5.9 ± 0.11c	35 ± 2.0a	SCL	24.6 ± 1.3b	1.08 ± 0.02d

CC, citrus control; CO, citrus orchard; GC, guava control; GO, guava orchard; LC, loquat control; LO, loquat orchard; SCL, sandy clay loam; SC, sandy clay; SL, sandy loam.
The data are displayed as means, ± values are standard errors. Small letters indicate significant differences at $p < 0.05$ between different treatment types.

TABLE 2 Changes in nutrients concentrations (mg kg^{-1}) in soils as influenced by three types of fruit orchards*.

Sites	Soil depth (cm)	N	P	Ca	Mg	K	Na
CC	00–25	224 ± 12g	1, 299 ± 23f	1, 284 ± 10g	1, 093 ± 16d	5, 282 ± 35g	574 ± 12g
	25–50	202 ± 15h	1, 133 ± 30i	1, 127 ± 12i	1, 100 ± 18c	5, 520 ± 30e	651 ± 15f
CO	00–25	279 ± 18e	1, 347 ± 33d	1, 444 ± 10e	1, 065 ± 24e	6, 649 ± 40a	706 ± 22e
	25–50	294 ± 10d	1, 259 ± 22g	1, 430 ± 17e	1, 062 ± 22e	6, 532 ± 32b	631 ± 16f
GC	00–25	226 ± 17g	1, 358 ± 21c	1, 317 ± 18f	878 ± 12h	4, 311 ± 32j	655 ± 20f
	25–50	226 ± 21g	1, 112 ± 14j	1, 390 ± 20e	899 ± 10g	4, 395 ± 26i	749 ± 12d
GO	00–25	318 ± 16c	1, 433 ± 21e	1, 847 ± 11c	1, 108 ± 18c	5, 147 ± 26h	779 ± 18d
	25–50	294 ± 16d	1, 183 ± 26h	1, 744 ± 13d	1, 089 ± 12d	5, 440 ± 25f	813 ± 18c
LC	00–25	221 ± 24g	1, 016 ± 27k	1, 319 ± 13f	1, 087 ± 22d	4, 316 ± 34j	763 ± 21d
	25–50	252 ± 13f	1, 026 ± 22k	1, 147 ± 19h	977 ± 25f	4, 147 ± 29k	815 ± 19c
LO	00–25	459 ± 12a	1, 557 ± 24a	2, 440 ± 21a	1, 475 ± 20a	6, 440 ± 31c	973 ± 14a
	25–50	434 ± 10e	1, 333 ± 20e	2, 391 ± 22e	1, 398 ± 20e	6, 280 ± 30d	874 ± 18e

*CC, citrus control; CO, citrus orchard; GC, guava control; GO, guava orchard; LC, loquat control; LO, loquat orchard.
The data are displayed as means, ± values are standard errors. Small letters indicate significant differences at $p < 0.05$ between different treatment types.

$CaCl_2$ extractable P concentrations in soils sampled from these orchards were 212, 228, and 242 mg kg^{-1}, respectively. Mehlich 1 P was higher in the soil of loquat followed by guava and citrus orchards. Olsen P differed in the order of guava < citrus < loquat orchard. Bray 1 P was enhanced in the surface soil by 6.8, 26.2, and 119.2% in the fields of citrus, guava, and loquat orchards, respectively, when compared to control fields (Figure 5A). Irrespective of the extraction method, subsurface soils gave a lower amount of extractable P than surface soils in all fruit orchards. Extractable P of HCl in soil was increased from 452 to 487 mg kg^{-1} by citrus plants, from 447 to 528 mg kg^{-1} by guava, and from 452 to 549 mg kg^{-1} by loquat orchard as compared to control soil. Similarly, DTPA extractable P concentrations were also higher in soil under fruit trees as compared to control soil (Figure 6). Among the soils, the loquat orchard substantially increased the extractable P in the soils as compared to other orchards. This could indicate that P accumulation in soil occurred due to the addition of P from the plant litter. The extractable P was highly correlated with the SOM, soil texture, and CEC of soils. This also suggested the transformation of organic P into inorganic form in organic matter due to microbial activities. The correlation matrix showed that most of the extraction methods were correlated positively (Table 4). The result indicates that the ability of P extraction was different for different methods, but the pattern of P release from soil was similar. This is in agreement

TABLE 3 Changes in trace elements concentrations (mg kg^{-1}) in soils as influenced by three types of fruit orchards*.

Sites	Soil depth (cm)	Zn	Cu	Mn	Fe
CC	00–25	87.7 ± 5.6a	23.9 ± 2.3g	98.7 ± 7.8d	189.9 ± 12.5e
	25–50	65.7 ± 4.9d	34.5 ± 3.4d	86.8 ± 6.5e	147.8 ± 9.6g
CO	00–25	68.9 ± 6.7d	37.9 ± 3.5d	188.5 ± 9.6a	217.9 ± 14.7d
	25–50	84.8 ± 5.4a	42.3 ± 3.8c	196.8 ± 8.7a	230.7 ± 16.5c
GC	00–25	76.6 ± 6.5c	27.9 ± 2.9f	79.0 ± 4.7f	176.8 ± 9.7f
	25–50	54.8 ± 6.0e	31.2 ± 3.1e	85.7 ± 7.0e	172.8 ± 13.4f
GO	00–25	62.7 ± 5.4d	45.8 ± 4.3c	168.8 ± 9.7c	218.9 ± 16.5d
	25–50	83.8 ± 4.7ab	51.8 ± 3.2b	176.4 ± 8.3b	224.3 ± 14.8d
LC	00–25	45.7 ± 3.4f	27.9 ± 4.3f	65.7 ± 6.4g	177.9 ± 14.0f
	25–50	55.7 ± 4.6e	32.1 ± 4.0de	76.8 ± 6.0f	184.0 ± 15.4e
LO	00–25	54.7 ± 4.7e	56.7 ± 4.3b	98.8 ± 7.3d	245.8 ± 17.8b
	25–50	65.9 ± 5.4d	64.8 ± 5.8a	102.9 ± 8.3d	258.7 ± 16.0a

*CC, citrus control; CO, citrus orchard; GC, guava control; GO, guava orchard; LC, loquat control; LO, loquat orchard.
The data are displayed as means, ± values are standard errors. Small letters indicate significant differences at $p < 0.05$ between different treatment types.

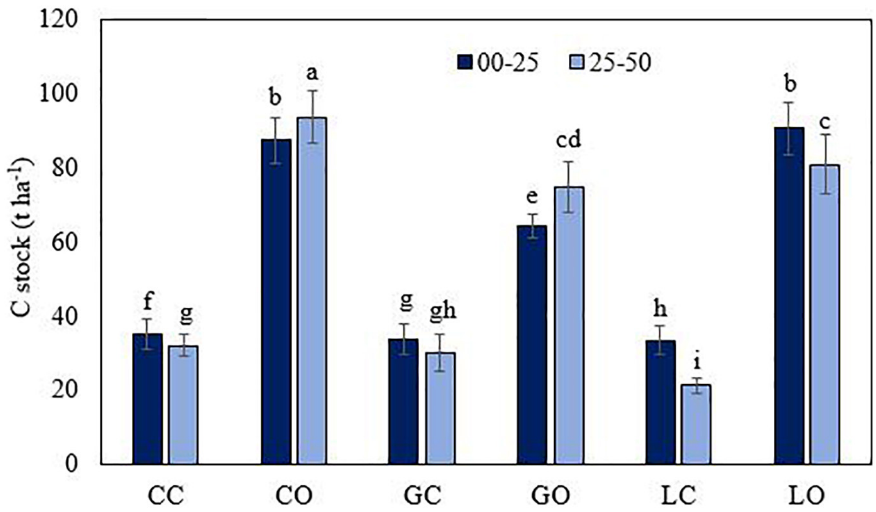

FIGURE 2
Carbon stock in soil (0–30 cm layer) as affected by three types of orchards. CC, citrus control; CO, citrus orchard; GC, guava control; GO, guava orchard; LC, loquat control; LO, loquat orchard. "±" Values are standard errors. Significant differences between the treatments at $p \leq 0.05$ are indicated by different letters.

with the findings of Haque et al. (2013). A summary of the two-way analysis of variance for orchards' effects on soil properties is given in Table 5.

4 Discussion

In total, seven extraction methods were compared for the extractability of P from orchard soils. Phosphorus amounts extracted through different extractants were varied in the order of HCl > DTPA > Mehlich 1 > Bray 1 > Olsen > $CaCl_2$ > water. Due to the various extraction procedures utilized for available P in the soil, these methods extracted varied P concentrations depending on the soil properties. The efficiency for the extractability of P among all extraction methods significantly differed in soils. This suggests that the extracting efficiency of the contrasting methods is strongly affected by the presence of available P in orchard-covered soils. The differences among the P extraction methods have been related to the extracting agents that may extract P fractions depending on the soil components involved in the P sorption process (Haque et al., 2013). The extractable P in soils was reported as Olsen > Mehlich-3 > Bray-1 by Haque

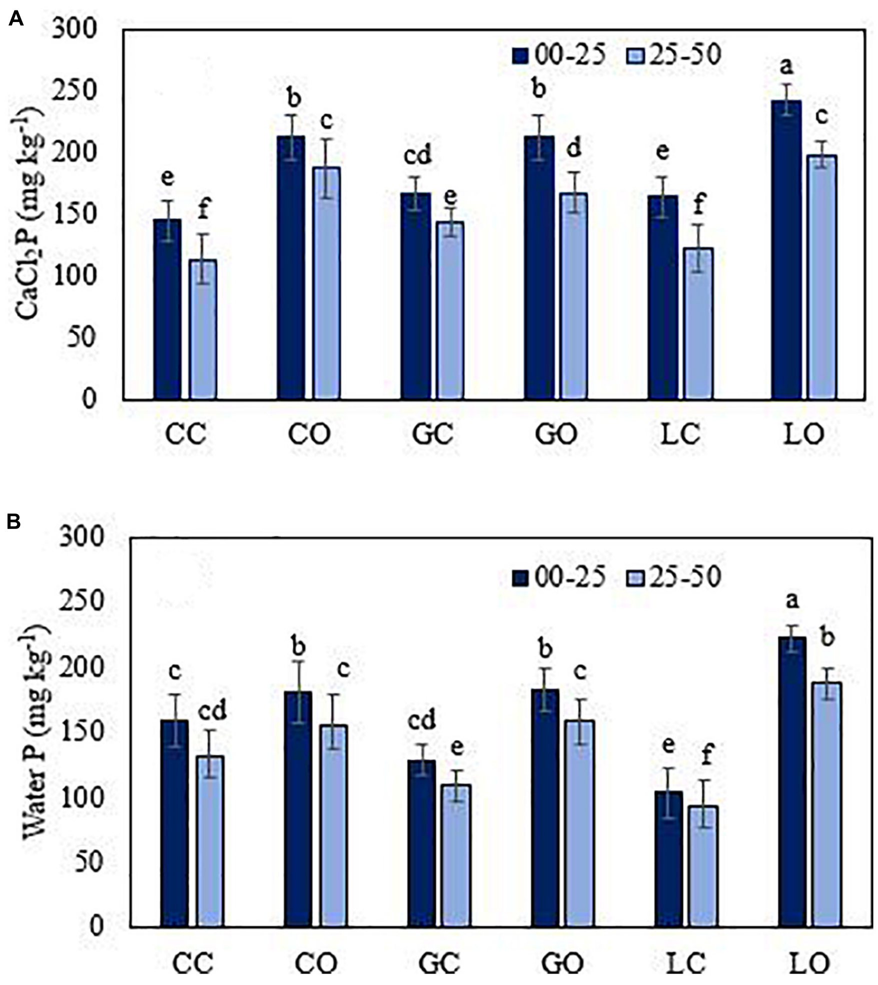

FIGURE 3
$CaCl_2$ **(A)** and water **(B)** extractable P from soil under three fruit orchards. CC, citrus control; CO, citrus orchard; GC, guava control; GO, guava orchard; LC, loquat control; LO, loquat orchard. "±" Values are standard errors. Significant differences between the treatments at $p \leq 0.05$ are indicated by different letters.

et al. (2013). Diversified methods (i.e., acid oxalate, Bray, and Mehlich 3 methods) have been utilized to analyze available soil P, due to the different soil conditions and their properties as well as different cropping patterns (Khaledian et al., 2018). The extraction methods based on water solubility and ion exchange (using neutral salt solutions) have been considered weaker for the P extraction from soils at pH 6, where P retention in soils was relatively low, whereas calcium acetate lactate, Mehlich 3, Bray II, dithionite, oxalate, and total P methods were reported as the strong P extraction methods for calcareous soils (Zehetner et al., 2018).

In this study, higher levels of inorganic P (Pi) were released from the soil samples of the loquat and citrus orchards. These differences in the P extraction from soils are largely attributed to the biomass pattern of trees, soil fertility, and controlling factors of P transformation in the soil. The occurrence of extractable P in soils may depend on the quantity and quality of leaf litter fall. This could predict that the quantity and quality of litter fall from orchards have affected the decomposition rate of litter and play a significant role in the P dynamics in soils. Wuenscher et al. (2015) also reported the differential extractability of P with different extraction methods and soil properties. Each extracting agent has a different ability to extract the amount of soil P because the reagent is targeted for different pools of soil P (Zhang et al., 2004). Phosphorus in soils may transform and be partially available, while the rest amount of P may either adsorb or precipitate in soils. The presence of total amount of P may not guarantee its availability in the soil. Ahmad et al. (1999) reported that in soils, P may be fixed on the surface of Ca/Mg carbonates as well as in the Ca/Mg phosphate compounds and converted the presence of P contents into insoluble or less soluble and thus retard the P bioavailability. Zhao et al. (2007) reported that the mineralization of organic P and the decomposition

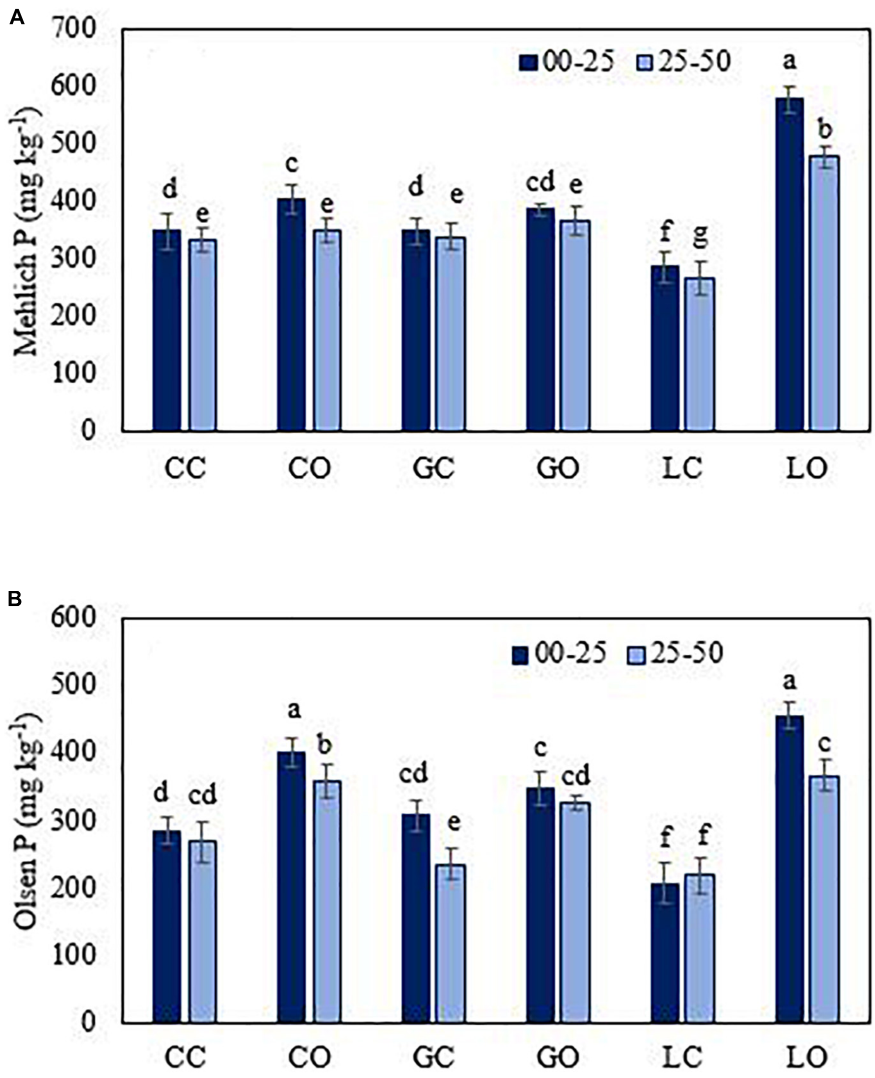

FIGURE 4
Mehlich 1 **(A)** and Olsen **(B)** extractable P from soil under three fruit orchards. CC, citrus control; CO, citrus orchard; GC, guava control; GO, guava orchard; LC, loquat control; LO, loquat orchard. "±" Values are standard errors. Significant differences between the treatments at $p \leq 0.05$ are indicated by different letters.

of litter were the main sources of available P and associated biological processes.

The available P could be predicted using a number of relatively stable soil properties such as soil texture and soil properties that could change over time periods, i.e., pH, extractable Ca, and total organic carbon (Khaledian et al., 2018). This makes a rapid assessment of P availability. The extractability of P was well correlated with clay percentage and organic matter contents in the soils. Soil texture affects the chemical characteristics of soil, including the formation of Al-organic matter bonded stable P and the leaching of P from soils (Sugihara et al., 2012), which may be related to the available P. Clay content was significantly related to the amount of P in the tested models because P can adsorb on the surfaces of clay minerals (Shen et al., 2011). Soil texture is important in determining P leaching from soils and influence the hydrology of soil (Negassa and Leinweber, 2009). The values of extractable P were related to the total amount of soil P. When the different extraction methods were correlated with each other, this showed that most extraction methods were correlated positively. However, the extent of correlation differs substantially among the P extraction methods used. Zheng and Zhang (2012) reported that texture and distribution of particle size heavily influenced soil P extraction in soils. This study revealed that the significant increase in soil P occurred under the influence of fruit tree species as compared to control soil (i.e., without an orchard). The differences in Pi concentrations in soil under long-term tree plantation may regulate P dynamics

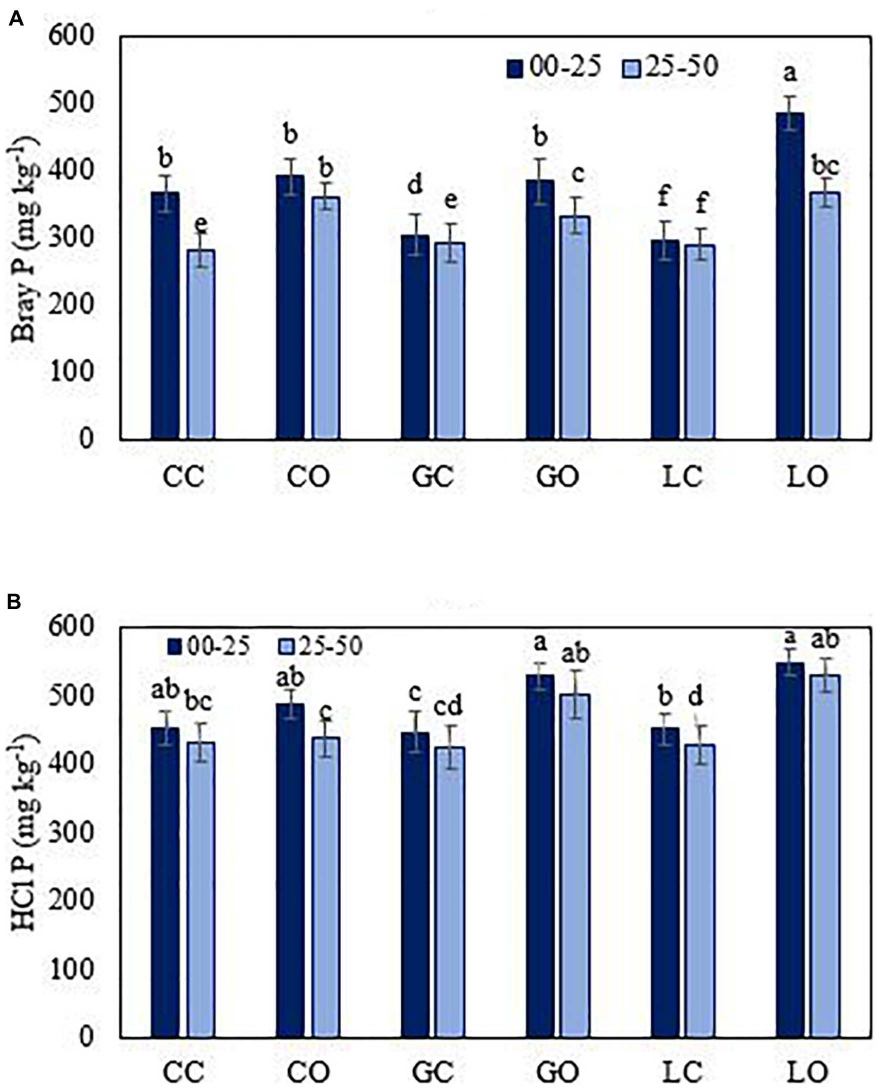

FIGURE 5
Bray 1 **(A)** and HCl **(B)** extractable P from soil under three fruit orchards. CC, citrus control; CO, citrus orchard; GC, guava control; GO, guava orchard; LC, loquat control; LO, loquat orchard. "±" Values are standard errors. Significant differences between the treatments at $p \leq 0.05$ are indicated by different letters.

in soils. Chen et al. (2008) reported enhanced mineralization of organic P and available P as a consequence of afforestation. Abdu (2006) also reported that the prevailing soil properties, i.e., pH and $CaCO_3$ content, soil texture, mineralogical composition, total surface area, organic matter content, and the presence of Fe and Al(hydr-) oxides substantially affected the solubility, availability, and extractability of P in the soil environment.

Soil organic matter has been considered an important component of soil quality. The amount of C in the soil was markedly affected by the presence of tree species. The C stock in soils differed by orchard species as loquat > citrus > guava. A higher amount of SOC was observed in the soil of a natural forest due to the higher density of the vegetation cover, deeper root distribution, greater size of the trees, larger biomass production, and litter inputs in the soil (Bakhshandeh et al., 2019). The extractable amount of P in the soil under the influence of different tree species followed almost a similar trend such as SOM. This indicates that total C contents in the soil could be linked to the P extractability in the soil. Organic matter contents in soil were also correlated with extractable P *via* seven extraction methods. Soil OC provides binding sites and influences P availability in soil (Kang et al., 2009), and changes in the organic P can regulate the P bioavailability from soils (Shen et al., 2011). Total P was well related to the amount of extractable P and clay contents, respectively. Yang et al. (2013) reported organic P as a major part of the soil P pool. A weak and

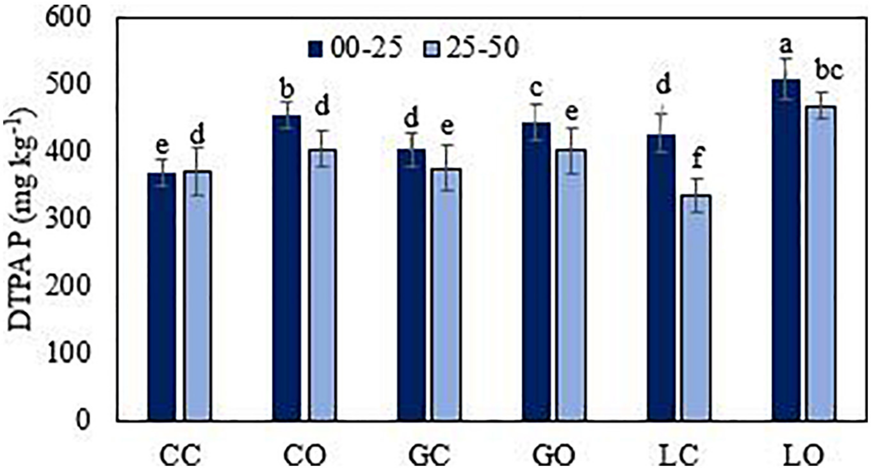

FIGURE 6
Diethylene triamine penta acetic acid extractable P from soil under three fruit orchards. CC, citrus control; CO, citrus orchard; GC, guava control; GO, guava orchard; LC, loquat control; LO, loquat orchard. "±" Values are standard errors. Significant differences between the treatments at $p \leq 0.05$ are indicated by different letters.

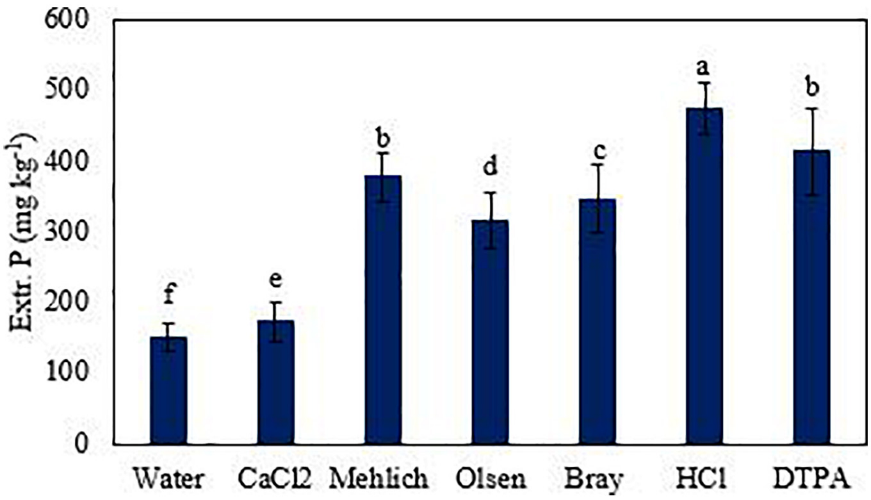

FIGURE 7
Extractable P across all soil samples (0–25 cm) using different extraction methods. Significant differences between the treatments at $p \leq 0.05$ are indicated by small letters.

inconsistent relationship of extractable P was found with the soil pH during the study.

The changes in the organic P indicated that organic matter inputs associated with tree growth influenced the P dynamics in soil (Chirino-Valle et al., 2016). Mehvish et al. (2011) reported the significance of macronutrients and micronutrients for the improvement of tree health, fruit yield, and quality. Various extraction methods have been reported for evaluating the availability of nutrients from soils (Ure and Davidson, 2002; Naidu et al., 2003). Menon et al. (1989) reported a positive relationship between P extracted with HCl and soil texture. Fernandes et al. (1999) reported that P extracted by Olsen, Fe-oxide Pi, and cation and anion exchange membranes was less dependent on the soil texture. Pueyo et al. (2004) recommended the 0.01 M $CaCl_2$ extraction method due to its feasibility for elemental analysis. This study inferred that fallen plant litter brought tangible changes in the P composition depending on the nature of the soil and the magnitude of SOM.

Plant nutrients were found in higher concentrations in orchards and the concentrations of K, Ca, and Mg in soil samples varied with the kind of plantation. Total K, Ca, and Mg were higher in soil afforested with loquat and citrus trees and lower in the soil sampled from guava. Sufficient amounts of these

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TABLE 4 Relationship among extractable P using different extraction methods and soil properties.

Parameter	Water	$CaCl_2$	Mehlich	Olsen	Bray	HCl	DTPA	Total P
$CaCl_2$	0.58*ns*							
Mehlich 1	0.91**	0.56*ns*						
Olsen	0.54ns	0.67*	0.65*					
Bray 1	0.64*	0.66*	0.68*	0.67*				
HCl	0.54ns	0.58ns	0.65*	0.75*	0.71*			
DTPA	0.63ns	0.64ns	0.70*	0.77*	0.81*	0.74*		
Total P	0.65*	0.57ns	0.74*	0.76*	0.87**	0.86**	0.89**	
SOM	0.76*	0.67*	0.78*	0.56	0.58ns	0.79*	0.83**	0.57ns
Clay	0.65*	0.67*	0.70*	0.50	0.57ns	0.63ns	0.87**	0.34ns
CEC	0.50ns	0.56ns	0.54ns	0.67*	0.60ns	0.66*	0.78*	0.65*
pH	−0.23ns	−0.27ns	−0.32ns	0.11ns	−0.33	−0.21ns	−0.30ns	0.11ns

The symbols "**" and "*" denote the levels of significance at p-value of 0.01 and 0.05, respectively, and ns denotes non-significance values.

TABLE 5 Summary of the two-way analysis of variance (ANOVA) for the effect of orchards on soil properties.

Parameter	Orchard (O)	Soil depth (S)	Interaction (O × S)
F-values			
Deionized water	17.31*	25.70*	ns
$CaCl_2$	80.20*	30.56*	ns
Mehlich 1	58.31*	52.78*	12.67
Olsen	51.72*	308.19*	65.49*
Bray 1	115.29*	36.78	24.22*
HCl	253.05*	928.07*	22.27*
DTPA	113.65*	2, 194.17*	15.41*
Total P	4838.6*	1, 878.87*	ns
SOM	16.89*	22.11*	ns
Clay	89.36*	6.92*	3.86*
CEC	10.86*	16.85*	6.10*
pH	0.67*	ns	ns

The symbol "*" denotes the level of significance at $p < 0.05$, ns denotes non-significance values.

nutrients in orchard soils could be attributed to the higher values of SOM (>1%) when compared to the control agricultural soil. This research indicated that the soil quality could be attributed to the type of orchard, growth pattern, amount of litter falls, and sequestration of SOM in the soil. Plant biomass production and nutrient cycling due to vegetation influenced the soil properties and plant nutrient availability (Chen et al., 2003). Different tree species have varying effects on nutrient cycling due to the differences in tree size, litterfall, and litter chemical composition (Mlambo et al., 2005). Zeraatpisheh et al. (2020) reported the spatial variations in soil properties and terrain attributes as a possible approach to delineating soil management zone for citrus plantations. Moreover, the higher correlation between soil properties and terrain attributes revealed considerable spatial variability, and a site-specific nutrient management system has been suggested (Zeraatpisheh et al., 2020).

The EC of orchard soils was found higher, which decreased significantly in control soils. An increasing trend in the soil EC was reported after the addition of organic materials of varying nature (Sarwar et al., 2003). Soils planted with orchard tree species were slightly more alkaline than those of adjacent control fields due to the higher concentrations of base cations. The increase in the base elements is likely to alter reactions on exchange sites, soil process rates, and the composition of the soil biotic community (Reich et al., 2005).

5 Conclusion

Land use has a significant influence on the available phosphorus (P). Loquat and citrus trees improved the soil quality more than guava species. SOM, CEC, and essential nutrients have been enhanced under the orchard system. Tree Plant species have varied effects on the P extractability from the soil. Hydrochloric acid (HCl) and diethylenetriamine pentaacetate (DTPA) extractants gave more amount of extractable P than other methods. Extractable P was markedly associated with the total amount of soil P. Topsoil showed more P concentrations than subsoil in all fruit orchards. Orchards have sequestered more amount of C stock than soil without an orchard. A higher amount of C stock in orchard soil as compared to control could be associated with higher inputs of leaf litter. The extractability P was well correlated with soil properties such as clay and organic matter contents in the soil. Therefore, we suggest the application of organic matter in soils for better orchard management. This study could help to address the issues related to P fertility and environmental management.

More studies are required on the P extractability in relation to the bioavailability using different extraction methods in contrasting soils for sustainable P management.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

MI, FU, QM, MS, and PA: experiment design. SB, MI, ZH, AA, and AH: soil sampling and experiment administration. MM, AN, MI, PA, FU, and QM: data analysis. SB, MT, MI, ZH, and MM: writing — original draft preparation, data verification, and proofreading. AN, NG, QM, and MS: proofreading. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Interactive effects of intercropping and mulching under conservation tillage as sustainable agriculture increased cotton productivity

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Climate change poses a significant risk to food security. Recent floods in Pakistan could serve as an example. In the current climate change scenario, there is a dire need to develop methods that increase crop productivity and reduce the threat of food insecurity in areas with low crop production. A detailed field experiment was conducted to check the effects of intercropping and straw mulching under conventional tillage (CT) and no tillage (NT) systems on soil health indicators and cotton productivity at the experimental area of Khwaja Fareed University of Engineering and Information Technology (KFUEIT), Rahim Yar Khan, Pakistan. The main plot treatments comprised CT and NT. The subplot treatments were sole cotton (C1), cotton + mung-bean intercropping (C2), cotton + mung-bean + straw mulching (C3) and cotton + straw mulching (C4) under CT, while sole cotton (N1), cotton + mung-bean intercropping (N2), cotton + mung-bean + straw mulching (N3) and cotton + straw mulching (N4) were the NT subplot treatments. Overall, NT increased plant height by 18.4 %, chlorophyll a and b contents by 28.2 and 21.1%, respectively, mean boll weight by 17.9%, and seed yield by 20.9% compared to CT ($P < 0.05$). The interaction of tillage and mulching increased plant height by 7.0% under CT and 21.8% under NT in comparison with no mulching. Similarly, straw mulching under NT increased chlorophyll a and b contents by 41.9 and 28.5%, respectively, mean boll weight by 26.9%, and cotton seed yield by 23.0% in comparison with no mulching under NT. Intercropping decreased crop yield without straw mulching but increased it under straw mulching. Further, straw mulching increased soil physicochemical properties under NT, which contributed to increasing crop productivity. We concluded that straw mulching under NT might be a promising practice for enhancing cotton yield, productivity, and soil health in low-productivity areas.

KEYWORDS

cotton-mung bean intercropping, straw mulching, conservation tillage, soil health, crop productivity, climate change

Introduction

The cotton crop, scientifically known as *Gossypium hirsutum* L., is cultivated throughout the world and is known as "white gold" (Puspito et al., 2015). It is an economically important crop that generates 600 billion USD annually (Shuli et al., 2018). The textile industry in Pakistan is dependent on cotton production, and Pakistan has great importance due to its large-scale cotton production every year; this places the country in the fourth position globally after India, China, and the USA on a global scale [Government of Pakistan (GOP), 2018]. Pakistan produced nearly 12 million bales on 2,699,000 ha, which helped raise the GDP by 1% through the agriculture sector (Ibrahim et al., 2008). On the other hand, Pakistan is among the most vulnerable countries that are highly susceptible to climate change events (Mehmood et al., 2022). Different factors, such as poor seed management, soil infertility, water scarcity, and expensive field treatments, play a major role in declining cotton yield and quality (Abdullaev et al., 2007).

Countries such as the Middle East and Australia face soil problems because of land changes, deforestation, and climatic conditions that have more detrimental effects in arid and semiarid conditions (Nosrati and Collins, 2019; Zeraatpisheh et al., 2020). Future strategies may focus on developing land for the agriculture sector within the populations of different communities without depleting natural resources (Broman and Robèrt, 2017). Sustainable agriculture is a fundamental part of making long-term plans for land development, as these strategies have low environmental hazards and better crop production (Busby et al., 2017). Sustainable agriculture is eco-friendly, less expensive, and protects the habitats that ensure security and conservation for plant and animal lives (Yadav et al., 2018). Soil microbial management (Jousset, 2017), minimum tillage (Singh et al., 2016), prevention of soil erosion (Bowers et al., 2020), soil fertility management, cover cropping, and intercropping are the main soil management practices (Martin-Guay et al., 2018; Sharma et al., 2018).

Agronomic factors such as tillage operating mechanisms, unsafe irrigation networks, and different seeding practices cause a reduction in crop production and quality (Farooq et al., 2020). Intense tillage practices to grow cotton deplete soil fertility and texture, resulting in poor crop production (Saharan et al., 2019). Soil erosion, nutrient depletion, and a lower water-holding capacity of the soil are all the results of single-crop repetition and traditional tillage techniques (Ryken et al., 2018). Enhancement of long-term crop production and yield depends upon conservation tillage that helps to improve soil quality (Martin-Guay et al., 2018). Previous studies revealed that conservation tillage techniques are suitable for arid and semiarid conditions where crop production is low, as these techniques help enhance phosphorus, nitrogen, potassium, and organic matter availability in the soil (Busari et al., 2015; Bhatt, 2017). Recent research suggested and proved conservation tillage is one of the fundamental and essential factors in increasing soil nutrients and soil quality (Duggan et al., 2005; Sayed et al., 2020). Long-term conservation tillage application boosts soil fertility by increasing micro soil biota (Page et al., 2020; Saha and Bauddh, 2020). Moreover, adding green mustard as manure increases organic matter accumulation in the roots of xerophytes in arid areas (Koishi et al., 2020).

Similarly, intercropping and cover cropping are advised for better quality and crop production, along with ensuring biodiversity restoration in an eco-friendly environment (Brussaard et al., 2007). Intercropping with leguminous plants is helpful for improving the economy, as these are cash crops and play a role in crop production (Baritz et al., 2018). Moreover, legumes have an important symbiotic relationship with nitrogen fixation bacteria, as the bacteria make root nodules, and they convert atmospheric nitrogen into different, useable forms by plants, which is called "biological nitrogen fixation." This useful relationship enhances soil fertility and crop production (Fustec et al., 2010). It also helps mitigate the danger of soil erosion (Hauggaard-Nielsen et al., 2008), enhancing moisture retention in the soil (Ghanbari et al., 2010), improving soil fertility (Hauggaard-Nielsen et al., 2009), and improving nutrient cycling and soil conservation (Chalka and Nepalia, 2006; Lithourgidis et al., 2011). Legume intercropping is a famous methodology for improving soil fertility and health, and many studies have described intercropping as the best strategy to increase the physical aspects of soil in recent years (Srinivasarao et al., 2012; Lal, 2015). We hypothesize that intercropping with straw mulching will improve cotton productivity and soil health under conservation tillage compared to the conventional tillage method.

Materials and methods

Site characteristics and climatic conditions

A short-term field experiment was conducted at an experimental area of Khwaja Fareed University of Engineering and Information Technology (28.4075 N, 70.3053 E, 86 m above sea level), Rahim Yar Khan, Pakistan, during the summer of 2022. The experimental site was located in a plain area with an arid climate under irrigated conditions. Long-term climatic data were taken from the district agricultural extension department by Rahim Yar Khan and are shown in Figure 1. Before conducting field experiments, < 15 and 15–30 cm deep soil samples were taken from each corresponding experimental unit and accurately analyzed to determine the different physicochemical properties of the soil profile. The physicochemical properties of the experimental site are given in Table 1.

Experimental details

The field experiment was laid out in a split-plot design under a randomized complete block design (RCBD) with three replications. The experiment comprised two tillage systems with a legume intercrop and straw mulching (32 experimental units): CT= conventional tillage, wheat residues incorporated; NT = no tillage, wheat residues retained; C1 = sole cotton; C2 = cotton + mung bean; C3 = cotton + mung bean + straw mulching, and C4 = cotton + straw mulching under conventional tillage. Similarly, N1 = sole cotton, N2 = cotton + mung bean, N3 = cotton + mung bean + straw mulching, and N4 = cotton + straw mulching under no-tillage. The plot size was 18 m^2 (6 m * 3 m), and each plot was separated from the others by a distance of 0.5 m.

Direct drilling of seedlings was used to plow NT plots. The current experiment used the CT plots from the long-term cropping pattern. In the CT system, experimental plots were prepared by plowing with a conventional disc harrow to a depth of 30 cm before being properly planked the soil to mix the wheat crop residues. However, NT plots had cotton seeds sown directly on the tilled soil after harvesting wheat by retaining 30–50% of the wheat crop residues. Weeds were manually pulled away. On April 20, 2022, the cotton variety CIM-573 was seeded with a tractor-mounted Kharif drill at a seed rate of 15 kg ha^{-1}.

Additionally, the cotton crop was intercropped with a cover crop (mung bean variety NM-2016) 20 days after sowing. A basal application of 90 kg ha^{-1} of P, 60 kg ha^{-1} of K, and one-third of the necessary nitrogen dose (total of 160 kg ha^{-1}) was made at the planting time. The remaining N was divided into three equal portions, each weighing 35.6 kg ha^{-1} and applied at the first, third, and fifth irrigations. The canal water was used to irrigate the crops at the designated irrigation schedule stages (the first irrigation was done 20 days after sowing and subsequent irrigations at 10–15 days intervals depending upon weather conditions and crop requirements). The initial soil parameters of each plot were assumed to be the same.

Measurements and analytical procedures

Phenological, physiological, and yield attributes of the cotton crop

The chlorophyll content was calculated using a chlorophyll meter (SPAD-502; Minolta, Tokyo, Japan). The mean boll weight (MBW) was measured by randomly selecting 10 bolls from each experimental plot, and plant height was measured from the base to the tip of the main stem. The seed cotton yield (kg ha^{-1}) was calculated by multiplying the seed cotton yield (kg/plot) from the net plot area with the seed cotton weight from the 10 previously harvested bolls.

$$\text{Seed yield (kg/ha)} = \text{Seed yield (kg/plot)} \times 10,000\text{m}^2 / \text{Net plot area (m}^2\text{)} \quad (1)$$

Plants were taken from a 1 m^2 area at maturity to measure the total biomass. The collected samples were sun-dried until the weight remained constant. Using the conversion factor, the sample dry weights were then converted to biomass (kg ha^{-1}). Furthermore, the following formula was used to calculate the harvest index (HI) given by Sharma and Smith (1986).

$$\text{Harvest index (\%)} = \text{Seed yield (kg ha}^{-1}\text{)} / \text{Biological yield (kg ha}^{-1}\text{)} * 100 \quad (2)$$

Determination of soil physicochemical properties

Five soil samples from each experimental unit (<15 and 15–30 cm depths) were taken and examined for the various physicochemical characteristics of soil in accordance with standard operating procedures. A saturated soil paste was made to measure the soil pH, and electrical conductivity and pH and electrical conductivity meter were measured using a pH meter and electrical conductivity meter, respectively. The soil samples were dried and put through a 2-mm mesh filter. Wet oxidation was used to assess the organic matter in the soil (Walkley and Black, 1934). The amount of N, P, and K present in the soil was calculated by using the alkaline potassium permanganate (Subbiah and Asija, 1956), sodium bicarbonate (Olsen, 1954), and ammonium acetate (Nelson and Heidel, 1952).

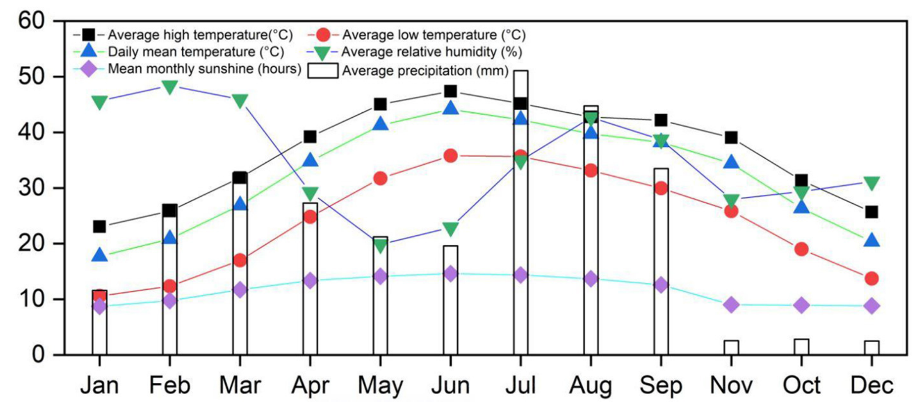

FIGURE 1
Long-term weather data (average high and low temperature, daily mean temperature, average relative humidity, mean monthly sunshine hours, and average precipitation at the experimental area.

TABLE 1 Soil physicochemical properties of the experimental site at cotton sowing and harvest under conventional tillage (CT) and no-tillage (NT) methods.

	Depth cm	ECe dS/m	pH	Organic matter (%)	AP (ppm)	AK (ppm)	Saturation %
At sowing							
CT/NT	<15	3.36	7.8	0.63	7.1	348	36
	15–30	2.55	7.8	0.56	4.2	215	38
At harvest							
CT	<15	3.35 a	7.7 a	0.66 b	7.3 b	349 b	36 b
	15–30	2.55 a	7.8 a	0.58 a	4.2 b	218 ab	39 ab
NT	<15	3.30 b	7.7 a	0.69 a	7.7 a	355 a	39 a
	15–30	2.51 b	7.8 a	0.58 a	4.4 a	219 a	40 a

Numbers followed by different letters within a column are significantly different at $P \leq 0.05$ by the least significance difference test.

Statistical analysis

For the statistical analysis, analysis of variance (ANOVA) was utilized for each of the study's parameters. To further differentiate differences between treatment means at a $p \leq 0.05$ as a significant threshold, Tukey's honestly significant difference (HSD) test was performed (Steel et al., 1997).

Results

Effect of management practices on plant height

The effects of intercropping and straw mulching on cotton plant height varied significantly under CT and NT. Figure 2 designates the effects of intercropping and straw mulching on cotton plant height under CT and NT. NT overall increased plant height by 18.4 % compared to CT ($P \leq 0.05$). The interaction of tillage with mulching and intercropping indicated significant impacts on plant height, such as straw mulching under CT (C4), which increased plant height by 7.0% compared to CT control (C1). Similarly, straw mulching under NT (N4) increased plant height by 21.8% compared to NT control (N1). However, intercropping decreased plant height by 7.2% under CT but increased it by 4.1% under NT compared to their respective control treatments. Mung bean intercropped with cotton under straw mulching (N3) increased plant height under NT ($P \leq 0.05$) but remained non-significant under CT (C3) (Figure 2).

Effect of management practices on chlorophyll contents

The effects of intercropping and straw mulching on cotton chlorophyll contents varied significantly under CT and NT, with NT significantly increasing chlorophyll a and chlorophyll

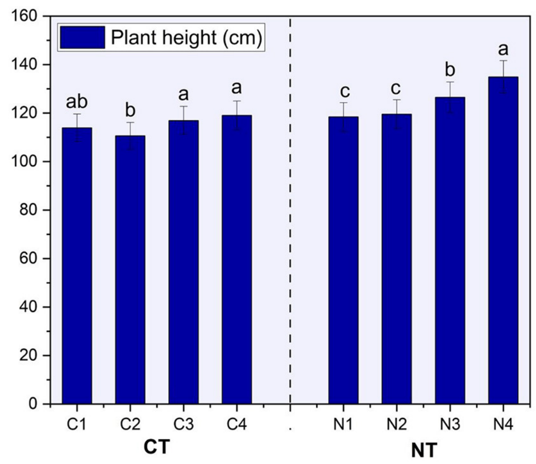

FIGURE 2
Interactive effect of intercropping, mulching, and different tillage systems on plant height. Different letters above graph bars indicate significant differences $p \leq 0.05$).

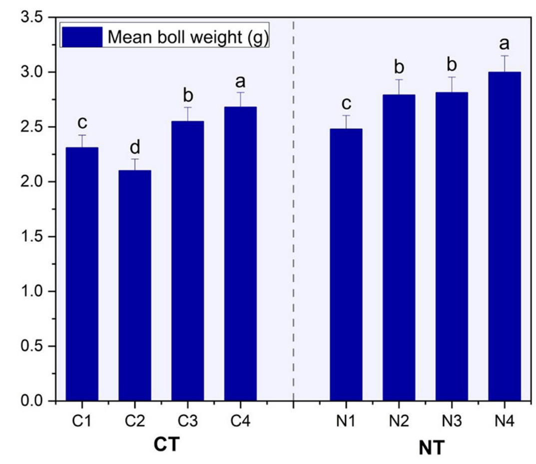

FIGURE 4
Interactive effect of intercropping, mulching, and different tillage systems on the mean boll weight of cotton. Other letters above graph bars indicate significant differences at $p \leq 0.05$).

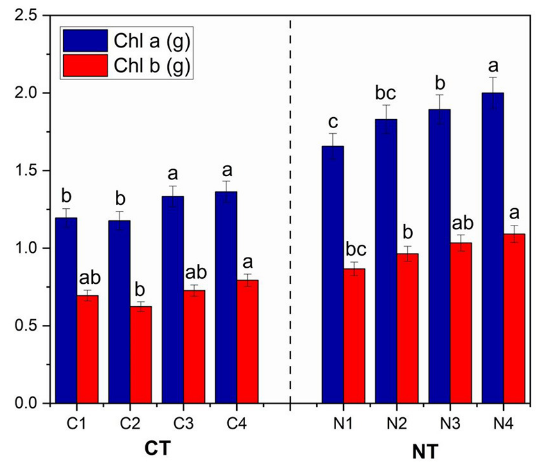

FIGURE 3
Interactive effect of intercropping, mulching, and different tillage systems on chlorophyll a (Chl a) and chlorophyll b (Chl b) contents. Other letters above graph bars indicate significant differences at $p \leq 0.05$).

b contents by 28.2 and 21.1%, respectively, compared to CT (Figure 3; $P \leq 0.05$). The interaction of tillage with mulching and intercropping indicated significant impacts on chlorophyll contents, such as straw mulching under CT (C4), which increased chlorophyll a and chlorophyll b by 16.8 and 7.7%, respectively, compared to control (C1). Similarly, straw mulching under NT (N4) increased chlorophyll a and chlorophyll b by 41.9 and 28.5%, respectively, compared to NT control (N1). However, intercropping under CT (C2) decreased chlorophyll a and chlorophyll b contents by 3.2 and 8.2%, respectively, compared to control C1. In contrast, intercropping under NT (N2) increased chlorophyll a and chlorophyll b by 24.9 and 17.2%, respectively, compared to control N1. Mung bean intercropped with cotton with the application of straw mulching (C3) significantly increased chlorophyll a and chlorophyll b contents by 14.8 and 9.5%, respectively, compared to C1. Similarly, N3 significantly increased chlorophyll a and b contents by 21.8 and 16.5%, respectively, compared to N1.

Effect of management practices on mean boll weight

The effects of intercropping and straw mulching on cotton mean boll weight varied significantly under CT and NT. Figure 4 shows the effect of intercropping and straw mulching on cotton for mean boll weight under CT and NT. NT significantly increased mean boll weight by 17.9% compared to CT (C1) ($P \leq 0.05$). The interaction of tillage with mulching indicated significant impacts on mean boll weight, such as straw mulching under CT (C4), which increased mean boll weight by 21.0% compared to CT control (C1). Similarly, straw mulching under NT (N4) increased cotton mean boll weight by 26.9% compared to NT control (N1). However, intercropping under CT (C2) decreased mean boll weight by 13.1% compared to control (C1) but increased it by 17.7% under NT (N2) compared to control N1. The mung bean intercropped with cotton with the application of straw mulching (C3) increased mean boll weight by 12.4% and, under N3, by 21.1% compared to their control treatments.

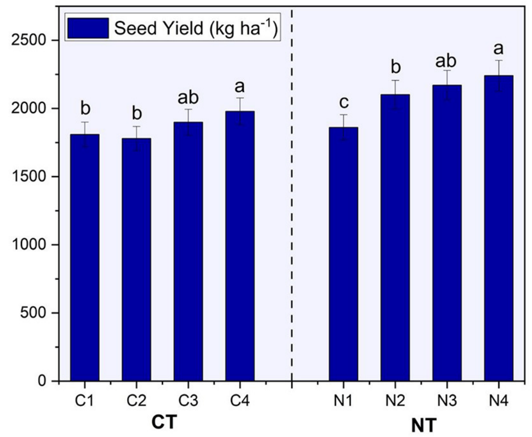

FIGURE 5
Interactive effect of intercropping, mulching, and different tillage systems on cotton seed yield. Other letters above graph bars indicate significant differences $p \leq 0.05$).

Effect of management practices on seed yield

The effects of intercropping and straw mulching on cotton seed yield varied significantly under CT and NT, such that NT significantly increased seed yield by 20.9% compared to CT ($P \leq 0.05$) (Figure 5). The interaction of tillage with mulching indicated significant impacts on seed yield, such as straw mulching under CT (C4), which increased seed yield by 12.9% as compared to CT control (C1), while straw mulching under NT (N4) increased seed yield by 23.0% as compared to NT control (C4). However, intercropping decreased seed yield by 4.1% compared to control C1 under CT but increased it by 20.7% under NT compared to control N1. Mung bean intercropped with cotton under straw mulching significantly increased seed yield by 8.4 and 9.1% under NT and CT, respectively. Moreover, NT increased the harvest index (HI) by 0.6% compared to CT.

Soil health indicators

Indicators of soil health such as soil organic matter (SOM), pH, ECe, and soil available P and K are summarized in Table 1. The employment of diverse tilling methods, legume intercrops, and mulching had a significant impact on these indicators. NT enhanced soil organic matter by 9.52%, available potassium (AK) by 2.0%, available phosphorous (AP) by 8.45%, and saturation percentage by 8.33% in $<$ 15 cm of soil. NT enhanced soil organic matter by 3.57%, available potassium by 1.86%, available phosphorus by 4.76%, and saturation percentage by 5.26% in the top 15–30 cm of the soil layer. However, NT decreased soil ECe by 1.36% in $<$ 15 cm and 1.57% in 15–30 cm of soil.

There was a strong correlation between mulching, legume intercropping, soil health indices, and a couple of modes of tillage. There was a positive Pearson relationship between soil organic matter, soil availability of phosphorus and potassium, seed production, biological yield, and harvest index (Figure 6).

Discussion

The overall phenological stages were smoothly completed by the cotton crop grown under NT, with the results showing better overall production under NT than CT. It is comparable to the earlier cotton study conducted using conservation tillage (Qamar et al., 2015). In comparison with the CT system, the NT system under straw mulching displayed greater values for the physiological characteristics of the cotton crop (Figures 2–5), as determined in earlier studies such as those described by Chakraborty et al. (2010), who compared no mulching to straw mulching and found that it increased crop yield by 13–25%, which might be because of the strongest root growth and development that strongly accounted for higher physiological characteristics and greater assimilation partitioning (Rajpoot et al., 2018).

In this study, a stronger connection between tillage and mulching was discovered for cotton physiological and yield parameters, such as crop yield, which increased with NT straw mulching compared to other combinations (Figure 5). This suggests that residue cover or reduced tillage with NT can store more soil moisture by reducing rainfall water loss and soil surface evaporation, which ultimately increases crop yield (Wang et al., 2011; Adil et al., 2022a,b). According to Wang et al. (2018), straw mulching enhanced precipitation storage efficiency by 13–16% compared to no mulching. Wheat production and soil water content both increased by 23%, while water usage efficiency (WUE) improved by 33% because of straw mulching (Zhang et al., 2015). Similarly, compared to no mulching, straw mulching increased crop grain yield by 13–25% (Chakraborty et al., 2010). According to numerous studies, a traditional tillage strategy improves the morphological and yield characteristics of the cotton crop in the first few years (Dhima et al., 2007). However, under NT, the yield of seed cotton increased because of enhanced soil porosity (Table 1 and Figure 6) (Qamar et al., 2015) and increased water use effectiveness (Rahman et al., 2018).

Due to favorable soil and other environmental factors, the sole cotton crops, C1 and N1, had better phenological traits (Figures 3–5) and recorded better physiological qualities than cotton-mung bean intercropping. This is due to the absence of competition for light, space, water, and nutrients, which promotes rapid growth and development and better phenological characteristics (Paul et al., 2013). Moreover, NT had appreciable variations in the current outcomes, such as higher plant height, mean boll weight, and seed cotton

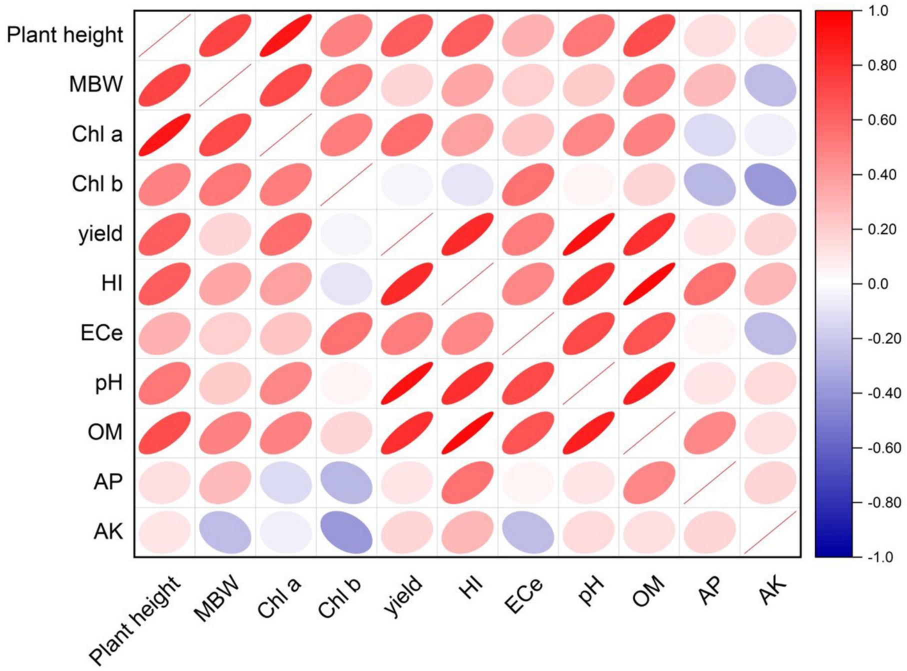

FIGURE 6
Correlation of growth, yield, and physiological attributes of the cotton crop, yield components of legume intercrops, and soil health indicators under different tillage systems and legume intercrops; the areas of circles show the absolute value of corresponding correlation coefficients tested at the * 0.01 significant level. PH, Plant height; TB, Total bolls per plant; MBW, Mean boll weight; Chl a, chlorophyll a; Chl b, chlorophyll b; Yield, seed cotton yield; HI, harvest index; ECe, electrical conductivity; pH, soil pH; OM, organic matter; AP, soil available phosphorus; AK, soil available potassium.

yield compared to CT, which significantly improved the morphological, yield, and yield-related characteristics of the cotton crop. This improvement may be attributable to higher root penetration into the soil and higher nutrient (N, P, and K) uptake to meet the needs of the cotton crop's growth (Ahmad et al., 2021).

According to our research, legume intercrops decreased plant height, total bolls per plant, and mean boll weight, which may be attributed to the competition for nutrients and water, space, and sunlight between cotton and mung bean, as previous studies reported decreased crop yield with cover cropping compared to no cover cropping in the first year of study (Zhang et al., 2015), instead of their ability to fix nitrogen (Chalka and Nepalia, 2006). The cover crops also tend to decrease ET (Adil et al., 2022a,b). The reason could be that lower soil water due to competition between cotton and mung bean could result in a less accessible soil moisture supply to the crop, reducing water evaporation from the soil surface and restricting crop transpiration, which could be the cause of the lowered ET caused by cover crops (Zhang et al., 2007).

In the present study, legume intercropping cultivated under the NT system had higher seed yield, higher biological output, and a higher harvest index compared to the CT system. Furthermore, it is clear that identical results under NT were reported in prior studies, such as Hou et al. (2012), who reported noticeable changes in crop production between conservation and conventional tillage systems. The grain yield improved with NT compared to CT by 9.6%, which might be due to improved soil physical and chemical characteristics that have been reported in previous studies (Fabrizzi et al., 2005). The reason could be that there is reduced soil disturbance, enhanced aggregate stability, and increased water-holding capacity under conservation tillage compared to conventional tillage (Hillel, 1998). Furthermore, these processes are beneficial for conserving soil water during crop planting, protecting against brief droughts during the growing season, and boosting crop yield (Pikul and Aase, 2003; Verhulst et al., 2011).

Another factor controlling the results is the fertilizer application; crop yield may have increased due to the effective application of nitrogen under perfect seedbed conditions for ideal growth and development (Hauggaard-Nielsen et al., 2008; Ahmad et al., 2016). The most recent results of the experiment indicated that mung-bean intercropped with cotton set better values for yield and yield-related qualities, which might be because these beans are grown under ideal conditions for mung-bean growth and had higher nitrogen fixation (Ahmad et al., 2020). A possible solution that preserves soil health indicators in arid climates is conservation tillage (Bhatt, 2017), as it increases the amount of organic matter, phosphate, and potassium in the soil, which eventually improves soil health indicators (Duggan et al., 2005; Sayed et al., 2020). Additionally, soil organic matter, available soil phosphorus, and potassium were higher with NT due to better soil quality indices (Table 1). Similar results for greater soil organic matter under NT and leguminous intercropping were obtained in previous studies (Page et al., 2020; Saha and Bauddh, 2020).

Conclusion

There was a need to develop methods that help increase crop production under the current climate change scenario in low-productivity areas. We wanted to assess the interactive effects of possible management practices on the current area, such as the previous crop production, which was totally dependent on conventional tillage. The current study indicated that straw mulching under conservation tillage performed better in terms of phonological, physiological, morphological, and yield attributes. However, soil analysis revealed that no-tillage and leguminous crop intercropping improved soil health indicators. Moreover, the interaction of tillage, leguminous crop, and mulching showed a better response on seed yield and harvest index. However, intercropping decreased cotton yield, which might be due to the competition for the uptake of nutrients, including water; however, the effect was antagonistic under straw mulching. In conclusion, no-tillage and straw mulching could be recommended for achieving higher cotton crop productivity. More long-term research and field studies are needed to raise awareness of no-tillage and the role of leguminous crops in nitrogen fixation and sustaining soil health in cotton-growing areas.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

HL, MA, CZ, ZY, SL, ZQ, and JW conceived the research and review, drafted the manuscript, and finalized it. AM and MR helped improve the draft by providing valuable suggestions and information. All authors contributed to the article and approved the revised version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Longitudinal section cell morphology of Chinese fir roots and the relationship between root structure and function

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Introduction: The longitudinal section cell morphology of Chinese fir roots was studied to better understand the relationship between root structure and root function.

Materials: In this study, the adjusted microwave paraffin section method and the selected two sample transparency methods were used to process the Chinese fir roots and combined with the laser scanning confocal microscopy (LSCM) technique, the morphology of Chinese fir roots longitudinal section can be clearly observed in a short time. At the same time, the observation effect of the longitudinal section cell morphology of the LSCM image of the thick section of the Chinese fir roots and the ordinary optical imaging of the thin section was analyzed and compared.

Results and Discussion: The results showed that: (1) There were apparent differences in the observation effect of cell morphology in longitudinal sections of Chinese fir roots obtained using various treatment methods. Under LSCM, the section with a thickness of 20μm generated by the microwave paraffin section technique displayed complete cell morphology and clear structure in the root cap, meristem zone, and elongation zone. The overall imaging effect was good; the thickness was 0.42–1.01, 0.64–1.57, and 0.95–2.71mm, respectively. The cell arrangement in maturation zone cells was more regular. (2) Compared to the ordinary optical imaging of thin sections, the thick sections of roots made by the microwave paraffin section method shortened the time to obtain high-quality sections to ensure the observation effect. Therefore, adopting the microwave paraffin cutting approach to produce thicker root sections under LSCM allows for rapid observation of the cell morphology in longitudinal sections of Chinese fir roots. The current study provides the efficient operation procedure for the microscopic observation technology of the longitudinal section of Chinese fir roots, which is not only beneficial to reveal the relationship between the root structure and function from the microscopic point of view but also provides a technical reference for the anatomical study of other organs and the observation of the longitudinal section cell morphology of plant roots with similar structural characteristics.

KEYWORDS

Cunninghamia lanceolata, root tissue, cell morphology, microwave paraffin sections, laser scanning confocal microscope

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1. Introduction

Roots are critical functional organs for plants to uptake, transport, and store soil moisture and nutrients. It can respond to the combined effects of genetic characteristics and external environmental factors by regulating or changing its structural characteristics and morphological plasticity (Cabrera et al., 2022). The root cell morphology is the direct reflection of its development level. Its morphological structure can induce the tropism of root growth and extension and play an essential role in the growth and development of new roots (Hayashi et al., 2013). In addition, microstructural changes such as the thickening of the endodermis cell wall and suberification of the radial wall in the maturation zone of roots had significant effects on the selection of nutrients absorbed by roots (Scacchi et al., 2010; Yao et al., 2020).

At present, free-hand and paraffin sections are the widely used methods to observe the root anatomical structure. The hand-cut cross-section method is simple to operate, does not require fixation, dehydration, paraffin-immersion, and embedding, and is not treated with chemical reagents, which can significantly maintain the natural color and shape of the cells. However, when it is used to make the anatomical structure of longitudinal sections of the root system, the thickness is not well controlled, and the incision level cannot be guaranteed. This can easily damage the cell tissue structure (Wu et al., 2018). On the other hand, the paraffin section method uses paraffin to support cell tissue and turn it into a clear sheet, making it easy to observe changes in cell morphological characteristics (Mrak et al., 2021). However, there are often different degrees of bending in the development of longitudinal sections of the anatomical structure of the root system, particularly the root system of trees. The paraffin section technique makes it difficult to maintain cell alignment in longitudinal sections, leading to incomplete cell tissue structure (Wang et al., 2019). Additionally, the production procedure for this method is time-consuming, and the chemical reagents utilized in the sample processing and dyeing process can be dangerous to human health.

Researchers have optimized and improved every aspect of the paraffin section process, from selecting sectioning tools to the reagents used. Currently, the microwave paraffin section method, which uses microwave radiation heating to make polar molecules move at high speed to increase cell permeability and solvent molecule penetration, has become an effective method to shorten the pre-treatment time of paraffin section samples (Schichnes et al., 2001). The leaf anatomical structures of *Setaria italica* (Zhang et al., 2015), *Arabidopsis thaliana* (Inada and Wildermuth, 2005) and other plants produced by this method observed clear cell structures. Although the microwave paraffin section method can significantly shorten the pre-treatment time of samples, the later dyeing procedure is the same as the traditional paraffin section method. This process is cumbersome and has a low yield of excellent sections (Inada and Wildermuth, 2005; Wei et al., 2011).

The laser scanning confocal microscope (LSCM) is widely used in plant cytology, development, and histochemistry studies. It is based on fluorescence microscope imaging with a laser scanning device connected to a computer and related application software (Hasegawa, 2006; Wang et al., 2021). It has been used to observe the cell morphology of the roots' longitudinal section of plants such as *Pisum sativum* (Ropitaux et al., 2019) and *Camellia sinensis* (Sun et al., 2020). This provides a solution for optimizing the microwave paraffin section dyeing process. However, when the roots samples' thickness and hardness are more, light is challenging to penetrate the inside of the roots.

Consequently, the cell image on the computer will not be clear, the internal organizational structure will be blurred, and the complete cell morphology cannot be fully displayed. Due to lignin deposition, unequal hardness of each section of the tissue, and a high degree of lignification, it is not easy to detect a strong enough fluorescence signal inside the root system, particularly in trees (Barros-Rios et al., 2015). This has made it extremely difficult to clearly observe tree root cell morphology clearly. To meet the observation requirements under the LSCM, it is essential to appropriately transparent the tree root samples or to reduce their thickness.

Chinese fir [*Cunninghamia lanceolata* (Lamb) Hook] is an important timber tree species in southern China. Its roots have the characteristics of high lignification, large and uneven hardness in various parts. This study aimed to establish a method for precise and clearer observation of microscopic cell morphology in the longitudinal section of Chinese fir roots in a short time. This method is of great significance for improving the production efficiency of the longitudinal section anatomical structure of Chinese fir root samples and further understanding its growth characteristics. We hypothesized that the observed effect of Chinese fir root samples obtained by different treatment methods would differ. And compared to the transparent treatment of Chinese fir root samples directly, the effect of making root slices under the microscope would be more apparent and complete.

2. Materials and methods

2.1. Plant materials, experimental reagents, and apparatus

The plant seedlings of Chinese fir were from the 1-year-old No. 25 clone cultivated by the Chinese Fir Engineering Technology Research Center of the State Forestry and Grassland Administration. Late in July 2021, 60 young Chinese fir roots 5–7 cm long, well-grown, and free of pests and diseases were randomly selected and stored in formalin-acetic acid-absolute ethyl alcohol (FAA) for later use (Figure 1).

Experimental reagents include FAA (Formalin: Acetic acid: 70% Absolute ethanol = 1:1:18, v/v/v), PBS (0.1 mol·L^{-1} Phosphate Buffer Solution, pH = 7.2), PI (0.05 mg·L^{-1} Propidium Iodide, 0.1 mol·L^{-1} PBS was prepared), Safranine O-Fast green, TBA (Tert butyl alcohol), Benzyl benzoate, Benzyl alcohol, Xylene, Chloroform, and Absolute

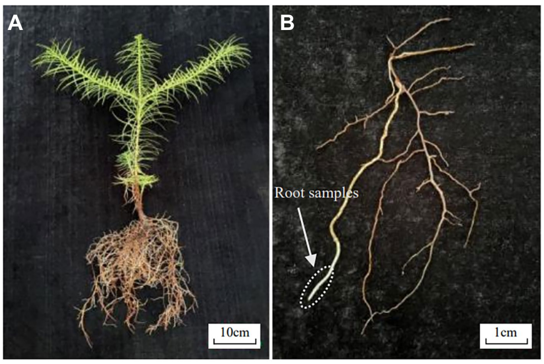

FIGURE 1
(A) Whole plant phenotype and **(B)** root materials of Chinese fir seedling.

ethanol. Experimental apparatus includes (1) ultra-high resolution and sensitivity laser confocal microscope (Carl Zeiss LSM 880, Oberkochen, Germany), (2) upright biological microscope (*Nikon* ECLIPSE E100, Tokyo, Japan), (3) full automatic rotary microtome (Leica RM 2265, Hessian, Germany), (4) Microwave [Galanz P70F23P-G5 (S0), Foshan, China], and (5) Diaphragm vacuum pump (Yuhua YH-500, Gongyi, China).

2.2. Methods for treatment of Chinese fir roots sample

The methods used in treating Chinese fir root samples are shown in Table 1, and the process flow chart is shown in Figure 2. The Chinese fir root samples were cut into 0.5 ± 0.1 cm long segments with a sharp blade and placed in a 10 ml centrifuge tube filled with FAA. Complete six segments for each method following the steps, ① fixed: centrifugal tubes containing Chinese fir root samples and FAA placed in a vacuum pump, vacuumized three times (pressure than 0.08 MPa), 10 min at a time, each time with a new fixative, after vacuuming, the fixative was replaced again and kept at room temperature for 12 h to fully fixation the material; ② dehydrated: After the fixation was completed, the fixative was sucked out, and gradient dehydration was performed according to the alcohol concentration of 70, 80, 90, 95, and 100%. Each gradient was treated for 45–60 min, and transparent treatment was performed after dehydration. Due to the different hardness and thickness of different plant tissue materials, the selection of transparent reagents and the duration needed for treatment had to be adjusted. This study made different transparent treatment schemes for Chinese fir root samples based on their lignification, hardness, and thickness. These schemes were based on traditional transparent treatment methods (Table 1, methods A, B, C, and D) to filter out the transparent treatment methods suitable for Chinese fir root samples. The dyeing was done following the transparency technique.

Method E treats Chinese fir root samples by microwave paraffin section after adjustment. The root samples were put in a centrifuge tube with FAA and pumped with a vacuum pump for 20 min. The fixative was then placed in a beaker with distilled water, set in a microwave, and set to heat preservation when the water temperature reached 70°C. The root samples were then placed in FAA and microwaved 3–4 times, each for 15 min. For dehydration, root samples were placed in 60% absolute ethanol, left at room temperature for

TABLE 1 The processing method of root system samples of Chinese fir seedlings.

Methods	Steps	Specific processing	Processing time (min)	Frequency of microwave processing	References
A	Transparency	Benzyl benzoate: Benzyl alcohol (2:1, v/v)	180–300	–	Zhao et al. (2021)
B	Transparency	Absolute ethanol: Xylene (2:1, v/v, 60 min) → Absolute ethanol: Xylene (1:1, v/v, 60 min) → Absolute ethanol: Xylene (1:2, v/v, 60 min) → Xylene (30 min) → Xylene (30 min)	240	–	Zhou et al. (2018)
C	Transparency	Absolute ethanol: Xylene (2:1, v/v, 120 min) → Absolute ethanol: Xylene (1:1, v/v, 120 min) → Absolute ethanol: Xylene (1:2, v/v, 120 min) → Xylene (30 min) → Xylene (30 min)	420	–	
D	Transparency	Absolute ethanol: Chloroform (3:1, v/v, 180–240 min) → Absolute ethanol: Chloroform (1:1, v/v, 180–240 min) → Absolute ethanol: Chloroform (1:3, v/v, 180–240 min) → Chloroform (90–120 min)	630–840	–	Zhang et al. (2017)
E	Fixation	FAA	15	3–4 times	Wei et al. (2011)
	Dehydration	60% Absolute ethanol	Stand at room temperature for 10 min	–	
		Absolute ethanol: TBA (1: 1, v/v)	15	1	
	Transparency	TBA	15	2	
	Paraffin-immersion	TBA: Paraffin (1: 1, v/v)	15	1	
		Paraffin	15	4	

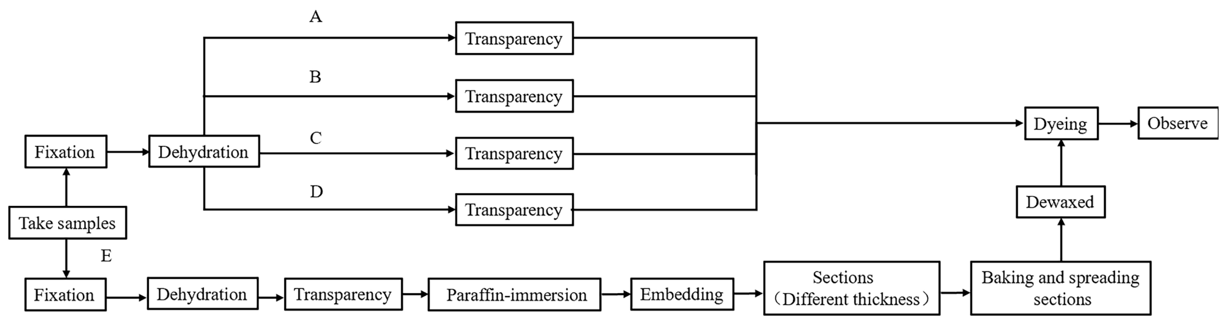

FIGURE 2
Flow chart of root system samples treatment of Chinese fir seedlings.

TABLE 2 Dewaxing and rehydration process.

Steps	Specific processing	Processing time (min)
Dewaxed	Xylene (20 min) → Xylene (20 min)	40
Rehydrated	Xylene: Absolute ethanol (1:1, v/v, 5 min) → 100% Absolute ethanol (2 min) → 95% Absolute ethanol (2 min) → 90% Absolute ethanol (2 min) → 80% Absolute ethanol (2 min) → 70% Absolute ethanol (2 min) → 60% Absolute ethanol (2 min) → 50% Absolute ethanol (2 min)	19

10 min, then placed in a mixture of absolute ethanol and TBA (1:1, v/v) and microwaved for 15 min. For transparency, root samples were placed in pure TBA and treated twice using a microwave for 15 min at each trial. For paraffin immersion, the root samples were placed in TBA: paraffin (1: 1, v/v) and microwaved once for 15 min. Following that, the new pure paraffin was replaced. The microwave treatment was repeated four times, each time for 15 min, to ensure that the paraffin was well absorbed in the root samples. The entire microwave treatment process maintained the water temperature at 70°C. Finally, it was embedded in paraffin. The rotary microtome was used in sections with different thicknesses (10 m, 20 m) to compare the imaging effect of thick sections under LSCM and conventional thin sections under an ordinary optical microscope. After sectioning, the paraffin tape was wrapped, dewaxed, and rehydrated (Table 2). Finally, the dyeing treatment was applied to the material.

2.3. Dyeing treatment

The dyeing procedures for PI and the other three conventional reagents (Safranine O-Fast green, Toluidine blue, and Hematoxylin) are shown in Table 3. In this study, the PI fluorescent dye was utilized for staining by the characteristics of the fluorescence signal of the tissue sample received by the LSCM. The prepared root samples and sections (20 μm) were placed in PI for staining at room temperature in the dark. The remaining PI was washed away by rinsing them thoroughly in PBS. Safranine O-fast green was used to dye the thickness sections (10 m) simultaneously.

2.4. Observation and photography

The stained root samples and sections (20 μm) were observed and photographed by LSCM. The excitation light wavelength was 561 nm. Chinese fir root samples were scanned layer by layer at a thickness of 4 m until the entire root sample was scanned. Simultaneously, the ScopeImage 9.0 (Nanjing, China) software was used to measure the cell morphological parameters of the functional areas (root cap, meristem zone, and elongation zone) of the longitudinal section of the Chinese fir root. The thickness sections (10 μm) were observed and photographed using an upright biological microscope.

3. Results

3.1. Observed effect of LSCM

The conventional transparent treatment methods are difficult to apply to the root tissue samples of Chinese fir. In this experiment, the transparent treatment scheme of Chinese fir root tissue samples was screened according to the characteristics of tree roots. The results showed that the transparency effect of method A (Table 1) was poor when the transparent treatment time was only 180 min. This was because tissue samples were not utterly transparent because of the short transparent treatment time. Longer treatment times with absolute ethanol and xylene and chloroform combined displacement (methods C and D in Table 1) resulted in severe hardening of Chinese fir root tissue and even shrinkage deformation. Finally, the root samples of Chinese fir treated with Method A (Treatment time 240 min) and Method B transparent treatment, which had better maintenance of original root morphology, were subjected to PI dyeing (Table 1).

The test samples were treated with the above two transparent methods A (Treatment time 240 min) and B. The results significantly differed when the LSCM was used (Figure 3). When the fixation and dehydrated steps were the same, after treatment with benzyl benzoate and benzyl alcohol as transparent reagents (Table 1, Method A, Treatment time 240 min), the fluorescence brightness of the root surface edge of Chinese fir was more potent than that of the root middle area. Still, no clear cell morphology was observed (Figures 3a_1,a_4). As the number of scanning layers increased by 3–5 layers, the apical and non-apical portions moved closer to the root center area, and the fluorescence intensity darkening range gradually increased (Figures 3a_1–a_7). Still, the clear cell morphology of the root's longitudinal

TABLE 3 Comparison of PI dyeing and conventional dyeing.

Reagents	Specific processing	Processing time (h)	References
PI	PI (10 min) → PBS (30 s) → PBS (30 s) → Stand at room temperature for 5 min	0.27	Lopez-Bucio et al. (2019)
Safranine O-Fast green	2% Safranine O (50% Absolute ethanol was prepared, 12 h–24 h) → 50% Absolute ethanol (2 min) → 60% Absolute ethanol (2 min) → 70% Absolute ethanol (2 min) → 80% Absolute ethanol (2 min) → 95% Absolute ethanol (2 min) → 0.2% Fast green (95% Absolute ethanol was prepared, 1 min) → 95% Absolute ethanol (2 min) → Absolute ethanol (2 min) → Absolute ethanol (2 min) → Xylene: Absolute ethanol (1:1. v/v, 2 min) → Xylene (2 min) → Xylene (2 min)	12.83–24.38	Chaffey (2000)
Toluidine blue	1% Toluidine blue (1% boric acid was prepared, 10 min) → Distilled water (2 min) → 50% Absolute ethanol (2 min) → 70% Absolute ethanol (2 min) → 85% Absolute ethanol (2 min) → 95% Absolute ethanol (2 min) → Absolute ethanol (2 min) → Absolute ethanol: Xylene (1:1, v/v, 2 min)	0.40	Sun et al. (2010)
Hematoxylin	1% Hematoxylin (5–10 min) → Flush with tap water (10 min) → Distilled water (3–5 s) → 95% Absolute ethanol (2 min) → 95% Absolute ethanol (2 min) → Xylene (5 min) → Xylene (5 min)	0.50–0.58	Gao et al. (2021)

section was not observed, indicating that the transparency of Chinese fir root samples failed to meet the observation requirements of LSCM.

When absolute ethanol and xylene were used as transparent reagents (Table 1, Method B), it was observed that under the same excitation light source conditions, compared with the apical portion surface, the longitudinal rectangular cell morphology in the edge area of the non-apical portion surface was clearly visible. Still, the complete and clear cell morphology was not observed in the middle area (Figures $3b_1$,b_4). Similarly, when 3–5 layers increased the number of scanning layers, the fluorescence intensity of both the apical and non-apical portions gradually decreased, and the darkening range gradually increased (Figures $3b_1$–b_7). This indicates that the Chinese fir root samples treated with this transparent reagent could not achieve the transparency observed under the LSCM.

Compared with the above two transparent processing methods, the anatomical structure made by the adjusted microwave paraffin section method (Table 1, Method E) can show the clear cell morphology of the longitudinal section of the Chinese fir roots under the LSCM (Figures $3c_1$–c_5). The root's functional areas, such as the root cap, meristem zone, elongation zone, and maturation zone, were clearly distinguishable. The size of the root cap cells was conical, the overall shape was irregular, and the arrangement was loose. The thickness of this functional area was 0.42–1.01 mm. A dome-shaped boundary distinguishes the root cap and meristem zone. The thickness of the meristem was 0.64–1.57 mm. The elongation zone moved to the maturation zone, the larger the individual cells were in rapid elongation, and the longitudinal size gradually increased (Figures $3c_1$,c_2). This functional zone was between 0.95 and 2.71 mm thick. The maturation zone was above the elongation zone. The cells in the maturation zone were regular rectangles; the individual cells were alike and can be seen in the epidermal and cortical structures of the tissue (Figures $3c_3$). In summary, this method can more clearly observe the cell morphology of the longitudinal section of Chinese fir roots than the transparent treatment of the sample.

3.2. Comparison of LSCM image and ordinary optical microscopy image

There are differences in the imaging effect of different thickness sections made by the microwave paraffin section method (Figure 4). The sections (10 μm thick) were better observed under an upright biological microscope. The cell morphology of the root cap, meristem zone, elongation zone, and maturation zone was identified (Figures $4d_1$,d_2). The sections (20 μm thick) had intact morphology and high identification of apical and non-apical portion cells under LSCM (Figures $4e_1$,e_2), and the functional areas of cells were clearly visible. As a result, clear cell morphology can be observed by LSCM and ordinary optical microscope for the longitudinal anatomical structure of Chinese fir roots with section thicknesses of 20 and 10 μm, respectively, in terms of imaging effect.

3.3. Comparison of treatment methods of Chinese fir roots sample

Significant differences exist in the processing time and operation steps of the cell morphology observation of different Chinese fir root's longitudinal sections (Table 4). The two transparent treatment procedures (Table 4, Methods A and B) go through four processes in 20 ~ 22 h for the same fixation and dehydration steps. Combined with the reagent treatment method of root samples in Table 1, it can be seen that Method A (Treatment time 240 min) has a less specific operation process than method B, however, the observation effect of method B is more ideal (Table 4). When the root samples were treated with the microwave paraffin section method (Table 4, Method E), the number of procedures was increased to 9. The complete and clear cell morphology of the longitudinal section of Chinese fir roots was observed in the anatomical structures of different thickness sections. However, under the same observation effect, the production of roots anatomical structure with a thickness of 20 μm combined with LSCM has fewer steps. It takes the least time, only 4–5 h, significantly improving production efficiency.

4. Discussion

The radial thickening growth stage of tree roots accumulates a considerable amount of lignin, and the degree of lignification is becoming increasingly evident; its cell wall is more solid than crop roots. The conventional paraffin section method requires the plant tissue to be softened for an extended period to resolve the sample's high density, hardness, and lignin content. However, the long-term softening treatment can easily cause the tissue to become brittle, and the cells appear empty or broken after sectioning (Moreno-Sanz et al., 2020). On

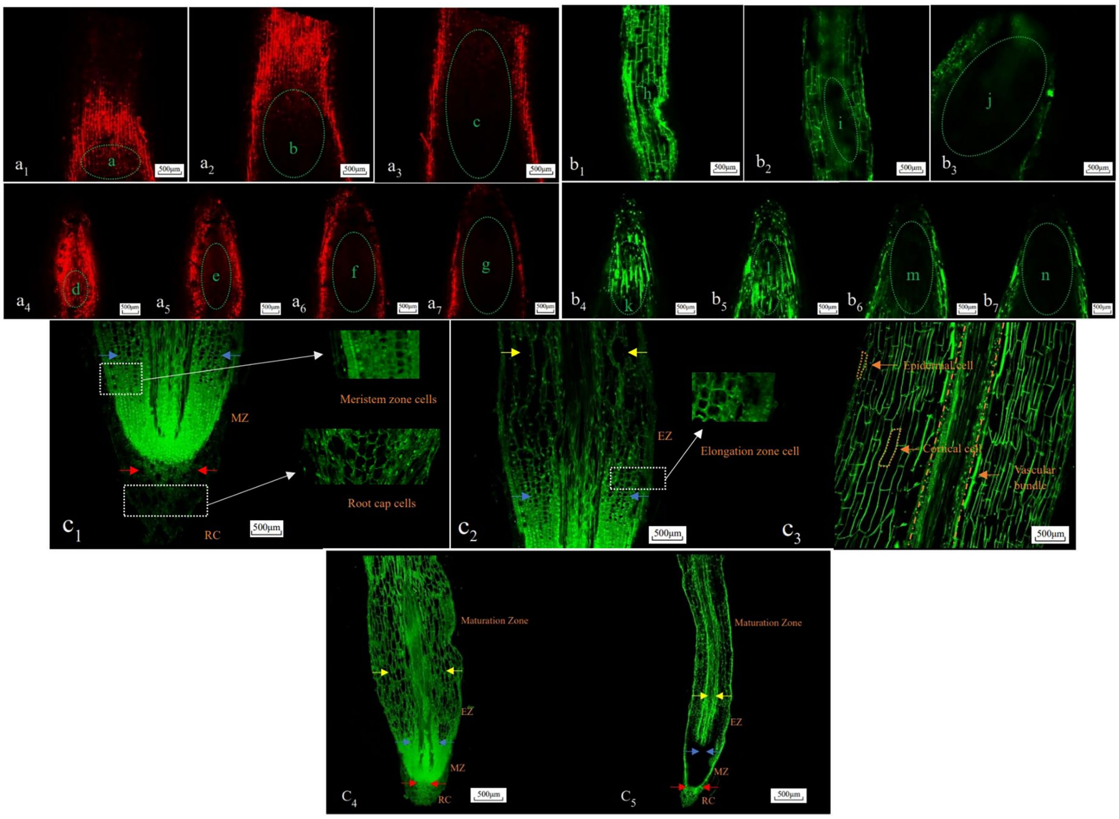

FIGURE 3
Longitudinal section cell morphology of Chinese fir roots under laser scanning confocal microscopy (LSCM) technique. a_1–a_7, b_1–b_7, c_1–c_5: Bar=500μm. a_1–a_3 and b_1–b_3: non-apical portion, a_4–a_7 and b_4–b_7 apical portion, a–n: middle root zone, c_1–c_3: longitudinal section anatomical structure of Chinese fir roots in microwave paraffin sections method, c_4,c_5: Vertical and bending longitudinal section anatomical structure of Chinese fir roots in microwave paraffin sections method (×10). RC, root cap; MZ, meristem zone; EZ, elongation zone; c_3 and above the elongation zone is the maturation zone, the red arrow represents the upper boundary of the root cap, the blue arrow represents the upper boundary of the dividing zone, and the yellow arrow represents the upper boundary of the elongation zone.

the other hand, microwave paraffin sectioning can drastically reduce sample processing time. However, different plant organs perform different functions in the environment, the degree of lignification varies, and the penetration rate and effect of extracellular solution vary. For example, under low-temperature stress, the content of soluble phenols in wheat (*Triticum aestivum* L.) roots decreased while the content of lignin increased, and the opposite trend was observed in leaf organs (Olenichenko and Zagoskina, 2005). Due to this, the microwave paraffin section method's final observation results were somewhat highly unclear. To achieve this, LSCM can directly monitor the samples with fluorescent labelling and sufficient transparency without a prolonged softening treatment, considerably reducing the risk of sample damage.

In the current study, Chinese fir root samples were made transparent using benzyl benzoate and benzyl alcohol, absolute ethanol and xylene, absolute ethanol, and chloroform as transparent reagents for different amounts of time. Methods A (Treatment time 240 min) and B did not show root hardening and deformation after treatment. However, under the LSCM, as the number of scanning layers increased, the root fluorescence signal intensity gradually decreased, and the internal cell morphology could not be clearly shown. This may be due to the natural growth and development of the roots of Chinese fir after the completion of primary growth. The oxidative polymerization of lignin monomers in the secondary xylem forms lignin and deposits on the cell wall. The lignification degree of most fine roots was also very high, and it gets firmer as it moves from the meristem zone to the root axis (Barros-Rios et al., 2015; Song et al., 2019). Even though the clear reagent could get through some of the surface cell tissue, it could not get through the more woody cells inside the root system. Finally, the Chinese fir's root system wasn't transparent enough, which led to pigment absorption and light scattering by cytoplasmic components. The LSCM did not detect a strong enough fluorescence signal throughout the observation; hence a clear cell structure was not observed (Donaldson et al., 2001). It can be seen that the directly transparent treatment of Chinese fir roots with high lignification and hardness makes it difficult to clealry observe root cell morphology under the LSCM. The types of transparent reagents and treatment time can be further explored.

Our study used the microwave paraffin section method to process tissue samples quickly and the laser scanning confocal microscope to scan and analyze samples point by point and layer by layer. As a result of extensive experimentation, a production method was devised that provided clear observation of cell morphology in a longitudinal section of the root system of the Chinese fir. Under a laser scanning confocal microscope, the sections produced with this technique generate clear and structurally complete images of cell morphology. This study used sections with a thickness of about 20 μm. The laser scanning confocal microscope was used to observe the optical "sections." To a certain extent, this study avoids the problem of aligning the longitudinal sections of partially curved roots after making them. This can effectively replace the mechanical sections and clearly observe the complete cell morphology of the longitudinal sections of Chinese fir roots.

Moreover, the anatomical structure of Chinese fir roots with a 10 μm thick section provided an excellent optical microscopic imaging effect and a complete structure. Clear structures of different functional areas of Chinese fir roots were observed under a microscope. However, it is easy to cause tissue material damage and force the original cell morphology to change by moving back and forth often when using this method to make sections (Barbosa et al., 2010). The serious lignification of Chinese fir roots greatly affected the light penetration and had a certain effect on the good color of the late dyeing solution (Barros-Rios et al., 2015). Furthermore, the dyeing process for safranine O-fast green dyeing solution and other conventional dyeing reagents requires multiple replacement solutions and moving sections, which can easily lead to tissue material shedding. An increase in xylene treatment steps on the final observation effect also has a certain impact. As a result, while creating the anatomical structure of the Chinese fir root system, utilizing a thicker slice and PI staining combined with the characteristics of LSCM can considerably reduce the possible damage of conventional paraffin sections during the sectioning and dyeing process. This method can ensure the observation effect and significantly shorten the production time. Moreover, conventional paraffin sectioning frequently uses safranine O-fast green, toluidine blue, and hematoxylin sections. However, these procedures are laborious and often include the use of chemical reagents, particularly xylene odor stimulation, a suspected carcinogen that is volatile. As a result, the integrity of the cell structure can easily be damaged (Premalatha et al., 2013).

As an ethidium bromide analogue, PI emits red fluorescence after being incorporated into double-stranded DNA. Depending on the excitation light source, it can dye both living and dead cells simultaneously. It is a regularly used cell fluorescent dye. It is frequently employed in the observation and study of the life activities of plant tissues and organs, such as herbaceous plant root hairs and pollen tubes. It can cause the observed samples to have strong fluorescence properties (Rounds et al., 2011; Kirchhoff and Cypionka, 2017). In this study, Chinese fir root samples were dyed with PI. Compared to the conventional paraffin section dyeing method, the process was reduced to four steps and only 16 min of processing time. The use of toxic chemical reagents was effectively reduced and decreased the harmful effect on the operators. The results also indicated that PI could be used as a good fluorescent dye in the morphological observation of Chinese fir root cells. Also, the PI dyeing process does not require the slice to be moved back and forth many times, which reduces the risk of tissue material shedding to some degree. At the same time, the combination of absolute ethanol and TBA was used as a dehydrating agent in the process of dehydrating root samples by microwave paraffin sectioning. TBA is a widely used dehydrating agent. It has a clear effect and does not make it easy to shrink or harden cell tissue (Baskin et al., 2014). After the treatment, the paraffin-immersion treatment can be performed directly. The process not only reduces the need for organic reagents but also makes the final tissue sample clearer.

Although Chinese fir roots have high lignification and large hardness compared with herbs such as rice (*Oryza sativa* L.) and *A. thaliana*, it is not conducive to observing their cell morphology clearly and completely using a single microwave paraffin section method or LSCM. However, combining the two technical characteristics can comprehensively observe the cell morphology in large quantities of Chinese fir roots in a short time. Plant cell research increasingly focuses

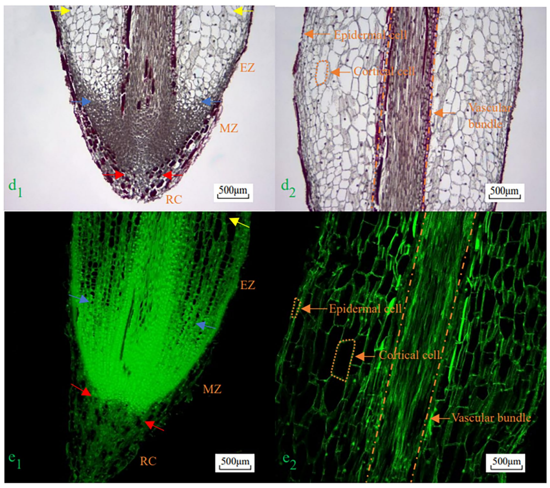

FIGURE 4
Morphological imaging effect of Chinese fir roots longitudinal section cells with different slice thickness. d_1,d_2, e_1,e_2: Bar=500μm, d_1,e_1: apical portion, d_2,e_2: non-apical portion, d_1,d_2: upright biological microscope, e_1,e_2: LSCM (×10). RC, root cap; MZ, meristem zone; EZ, elongation zone; d_2,e_2 respectively is the maturation zone, the red arrow represents the upper boundary of the root cap, the blue arrow represents the upper boundary of the dividing zone, and the yellow arrow represents the upper boundary of the elongation zone.

TABLE 4 Comparison of the observation preparation methods and effects of longitudinal section cell morphology of Chinese fir roots.

Methods	Transparent processing	Processing step	Time(h)	Observe effect
A	Benzyl benzoate and benzyl alcohol were used as transparent reagents	Fixation, dehydration, transparency, dyeing	20–22	The cell structure is not clear
B	Xylene and absolute ethanol were used as transparent reagents	Fixation, dehydration, transparency, dyeing	20–22	Complete and clear structure of some cells
E	Microwave processing	Fixation, dehydration, transparency, paraffin-immersion, embedding, sections, baking and spreading sections, dewaxed, dyeing	4–5 (Thickness 20 μm)	Complete and clear cell structure
			16.56–29.11 (Thickness 10 μm)	Complete and clear cell structure

on three-dimensional structure analysis of plant organs based on optical sections using LSCM three-dimensional reconstruction technology. For example, Hasegawa et al. (2016) used 200 optical sections to construct the three-dimensional structure of *A. thaliana* flowers and stems. Buda et al. (2016) reconstructed the cuticle of tomato (*Solanum lycopersicum* cv. M82) fruit using LSCM based on sequential optical sections and performed three-dimensional modeling. However, these techniques are rarely used in studying root growth and development of Chinese fir. The method described in the current study can be used to obtain root slices quickly. It is expected to reconstruct the three-dimensional configuration of the Chinese fir root in space using laser confocal technology to image its cell morphological structure as a whole, allowing for an in-depth study of the interaction between functional areas-environment or structure–function of Chinese fir roots.

In addition, LSCM technology is widely used in the study of plant life activities due to its improvement and perfection in the observation of ion (including Ca^{2+}, pH) changes in plant cells, organelles, and the cytoskeleton, as well as the process of plant growth and development and a particular protein signal transduction pathway (Pozhvanov, 2018). For example, Wang et al. (2016) used LSCM to find that the NO regulates the signal network, including Ca^{2+}, reactive oxygen species, and pH, during pollen tube development of *C. sinensis* under low-temperature stress. Therefore, compared to other microscopes, the benefits of LSCM in the research of microscopic plant graphics show its vast application potential in investigating the development mechanism of trees' underground roots. LSCM can be used to learn more about the biology of tree roots. For example, specific fluorescent probes can be used to label root-related hormones that are released when the tree is under stress. LSCM on a computer screen shows clearly how the hormone moves through the root (Li et al., 2016). When combined with related gene technology, the vital mechanism of tree roots' resistance to stress was also shown.

5. Conclusion

The microwave paraffin section method combined with LSCM can achieve clear observation of the cell morphology of the longitudinal section of Chinese fir roots in a short period of time. The anatomical structure of the longitudinal section of Chinese fir roots with a thickness of 20 µm was made by fixation, dehydration, transparency, and paraffin immersion. After PI dyeing, the tissue structure's complete and clear cell morphology was observed under an LSCM. To ensure the quality of observation, the production time and staining steps were significantly shortened and reduced. This method can also provide some technical reference for the observation and study of cell morphology of other tree roots longitudinal sections. Still, there are differences in hardness, water content, and maturity of different plants and parts of the same plant. Moreover, the time of microwave treatment needs to be adjusted accordingly to make the tissue complete and not deformed to meet the needs of scientific experiments.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

PW, XM, JL, XZ, and LL conceived and designed the experiment. LL, TZ, and XL conducted the study. LL and PW wrote the manuscript. PW, XM, and JL reviewed the manuscript and contributed to the discussion. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Role of different organic and inorganic amendments in the biofortification of iodine in *Coriandrum sativum* crop

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Iodine deficiency disorder (IDDs) is one of the most prevailing and common health issues in mountainous communities. An effective way to control the prevalence and emergence of IDDs in remote areas is to use iodized salt. However, recent studies indicated that iodized salt is mostly lost during the cooking process. The current study of iodine biofortification differed from the previous studies in two main aspects: it involved exogenous organic iodine (OI), and inorganic iodine such as potassium iodide (KI), added in the amended soils, which previous studies did not consider. Moreover, the translocation, transformation, and distribution of iodine from soil to plants are poorly understood in amended soil. Thus, identifying an effective management option to enhance iodine (I) bioavailability in nutrient-deficient soils is currently a significant challenge. Therefore, a greenhouse study was conducted to investigate the effects of organic and inorganic soil amendments on the uptake of different iodine sources in coriander crops. Results showed that applying an inorganic iodine source significantly enhanced the iodine edible part of the crop compared to the control (p<0.05). The application of soil amendments relatively improved iodine uptake by the coriander crop compared to the control. The highest iodine was found in crop tissues grown in wood ash-amended soil supplemented with KI (291.97μgkg^{-1}). The KI uptake was significantly higher than the OI (p<0.05). Compared to OI, a higher translocation factor (0.96) and distribution coefficient (3.51) were found for plants treated with KI. Thus, this study indicates that a suitable soil amendment can be a better option for iodine biofortification and that it can serve as an alternative to iodized salt in preventing IDDs.

KEYWORDS

iodine deficiency disorders, iodine uptake, amended soils, micronutrients, coriander

1. Introduction

Iodine (I) is an essential constituent of thyroid hormones and critical for the metabolism and normal functioning of the human body (Ujowundu et al., 2011). Iodine deficiency disorders (IDDs) are prevalent in many countries worldwide, affecting between 80 million to 2 billion people around the globe (Dasgupta et al., 2008; Szybinski et al., 2010). The deficiency symptoms can lead to endemic goiter, cretinism, and fetal abnormalities (Smoleń et al., 2016; Ligowe et al., 2021). The recommended daily allowance (RDA) of iodine for adults is µg d^{-1} with a tolerable upper limit of µg d^{-1} (WHO, 2007a,b; Patrick, 2008). I deficiency has increased more than fourfold over the past few years (Szybinski et al., 2010; Verduzco-Gallo et al., 2014; Lawson et al., 2015; Zia et al., 2015).

The most common method for mitigating I deficiency is iodized salt, a common supplement used to eliminate iodine deficiency (Vejbjerg et al., 2007). Despite the broad application of iodized salt, pathological symptoms related to inadequate I intake are diagnosed in about 38% of the world's population (De Benoist et al., 2008; White and Broadley, 2009). This is because the concentration of I in salt is highly variable and dependent on the level of iodization (Kalimbira et al., 2005). Losses of up to 20% can occur during production, processing, packaging, and transportation while cooking processes can contribute to an additional loss of 20% (Winger et al., 2008; Lawson et al., 2015). Adults are also advised to lower their daily salt intake to reduce risks of kidney damage and cardiovascular diseases (Piccone, 2011; Zimmermann, 2011).

One way to counter IDD is the optimization of I into the edible portion of crops through different crop and soil management strategies (Chilimba et al., 2012; Manzeke et al., 2014). Among these management approaches, biofortification emerged as a practical crop-based approach dealing effectively with mineral malnutrition by enriching crops and food products with bioavailable micronutrients (Lawson et al., 2015). Iodine uptake *via* edible crops has been reported as a cost-effective measure to combat IDDs in humans (Zhu et al., 2003). Crop plants can increase iodine absorption and accumulation in their edible parts when subjected to exogenous applications of different iodine sources (Comandini et al., 2013; Weng et al., 2014). Tonacchera et al. (2013) reported a noticeable increase of iodine in the urine samples of people who consumed biofortified vegetables. Moreover, 80% of the iodine in human and animal bodies was derived from the edible part of the crop/vegetable under natural conditions (Welch and Graham, 2005). In contrast, the bioavailability of iodine from food sources can easily be enhanced by up to 99% (Weng et al., 2014).

Soil geochemistry plays a vital role in iodine retention and conversion, as it primarily depends on the transfer of iodine from the atmosphere *via* precipitation. Scientists are interested in studying this atmospheric transport of iodine and the soil's ability to absorb it. Moreover, soils of mountainous areas are more prone to iodine deficiency due to topographical and soil characteristics (Von Oettingen et al., 2017). Edible crops grown in iodine-deficient soils are usually low in iodine concentration, and humans and animals consuming those crops face multiple IDDs. Plants can uptake and translocate the organic and inorganic iodine species (iodide and iodate) from the soil (Takeda et al., 2019). Applying iodine-rich organic (alfalfa, dulse) or inorganic (potassium iodide-KI) sources may enhance the concentration and retention of iodine in the soil.

Coriander (*Coriandrum sativum* L.) is a culinary, herbal, and medicinal plant of the Apiaceae family and is an important spice crop (Mandal and Mandal, 2015). It is commonly used as an ingredient in daily food preparation (Fahad et al., 2020). Coriander is primarily grown in Pakistan, Bangladesh, India, Russia, Morocco, Central Europe, and China (Sahib et al., 2013). Different parts of the plant are associated with various health and biological activities. Coriander has been used remedially for several gastrointestinal disorders (Sahib et al., 2013). Seeds of the coriander plant are used in several food items (Bhuiyan et al., 2009; Anwar et al., 2011). It has been reported that the concentration of iodine in edible parts of fresh coriander may reach a level of 2.28 mg kg^{-1} (Weng et al., 2013).

Previous studies analyzed the fate, accumulation, and transformation of iodine as a function of various organic and inorganic treatments for crops (Zhu et al., 2003). However, research reports on the uptake of iodine by edible crops have been poorly documented. Moreover, the bioavailability of iodine from soils after applying the selected amendment has not been well established. Therefore, the objective of this study was to investigate iodine uptake by coriander (*Coriandrum sativum* L.) applied with organic and inorganic forms of iodine after adding soil amendments, namely, sawdust, rice husk, charcoal, wood ash, and gypsum.

2. Materials and methods

2.1. Preparation of iodine fertilizer

In the current study, two exogenous iodine sources were used to evaluate their bioavailability to plants grown in amended soils. One source was an inorganic conventional iodine source, i.e., potassium iodide (KI), and the second was derived from seaweed and was organic in nature. The organic source of iodine was extracted from alfalfa (Medicago sativa) and dulse (Rhodymenia palmata) and was purchased commercially. According to Hou et al. (1997), the seaweeds contained 2–3% water-soluble iodine.

2.2. Experimental site and greenhouse experiment

The experiment was carried out from April to June 2020, in a 350 m^2 (7 × 50 m) greenhouse, at the Commission on Science and Technology for Sustainable Development in the South (COMSATS) University Islamabad, Abbottabad (34°11′57.19″N, 73°14′50.36″E). The greenhouse had a bow-roof shed design structure covered with 100 µm thick transparent polyethylene film. The air temperature ranged between 30.7 and 38.4°C, while the humidity was between 21 and 28%.

2.3. Chemical analysis of soil and greenhouse experiments

The soil used in the experiments was collected from the old vegetable production area of the Abbottabad district. The soil was sieved through a 3 mm mesh sieve, and its main agrochemical properties are listed in (Table 1). The methods for soil analysis have

TABLE 1 Properties of soil used for the study.

Parameters	Value
Sand (%)	75.6
Silt (%)	16.6
Clay (%)	7.8
Texture	Sandy loam
C ($g\,kg^{-1}$)	21.6
Moisture (%)	18.2
Iodine ($mg\,kg^{-1}$)	0.34
Exch. Ca ($mg\,kg^{-1}$)	76.3
Exch. Mg ($mg\,kg^{-1}$)	56.7
Exch. K ($mg\,kg^{-1}$)	156.0
Exch. Na ($mg\,kg^{-1}$)	35.6
CEC ($mg\,kg^{-1}$)	324.6
EC (1:5) ($uS\,m^{-1}$)	125
pH (1:5)	7.6

TABLE 2 Chemical composition of nutrient solution.

Macronutrient (mM)	Concentration (μM)
KNO_3	6
Ca $(NO_3)_2$	3.5
KH_2PO_4	1.33
$MgSO_4$ $7H_2O$	0.5
NaCl	0.48
$H3BO_3$	10
$MnSO_4$ H_2O	0.5
$ZnSO_4$ $7H_2O$	0.5
$CuSO_4$ $5H_2O$	0.2

been reported previously (Mohiuddin et al., 2019a). The soil was sieved through a 3 mm sieve and packed into a pot. The soil had a background iodine level of 0.6 $mg\,kg^{-1}$. Vegetables planted without iodine were used as control groups for comparison. Sawdust, rice husk, charcoal, wood ash, and gypsum were applied to the soil as amendments at the rate of 10 $t\,ha^{-1}$ (based on 2 million kg soil per plow layer in ha). Amendments were mixed in the column's topsoil (0–20 cm). Iodine was applied using an inorganic form, i.e., potassium iodide (KI), and an organic source.

The organic source of iodine [extracted from alfalfa (M*edicago sativa*) and dulse (*Rhodymenia palmata*)] was commercially purchased and applied to the soil. The iodine sources were fertilized in the soil at 50 $mg\,kg^{-1}$. I dose of more than 50 $mg\,kg^{-1}$ was reported as phytotoxic by Humphrey et al. (2019). The experiment was a factorial (6 × 2) (6 soil amendments × 2 sources of iodine) resulting in 12 experimental units and arranged into a randomized complete block design (RCBD) replicated three times under greenhouse conditions. The base fertilizer was applied, and the soil moisture was maintained at 50% of the maximum field water capacity. During the experimental period, deionized water was added regularly to keep the moisture stable. Seeds of coriander were germinated on wet filters for 2 days in the dark at 25°C after sterilization. To develop seedlings, the germinated seeds were subsequently transferred into quartz sand and cultivated on a nutritional solution (Table 2). After 8 weeks of development, the seedlings were transferred into pots with a homogeneous growth condition. Each treatment was performed in triplicates. Vegetable tissues from the root, stem, leaf, and fruit were separately collected after harvest. They were then dried with absorbent paper after being washed with deionized water. The fresh weight of each part was measured before slicing it into smaller pieces. These were then dried at 50°C, and pulverized in a stainless steel mill. The tissue samples were homogenized after sieving through a 60-mesh sieve and then kept at 4°C until analysis.

2.4. Iodine determination

Iodine in soil and plant was measured by methods used by Kesari et al. (1998). For the experiment, 0.5 g of sieved soil r was mixed with 15 ml of deionized water, shaken for 30 min, and filtered *via* filter paper Whatman 42. The filtrate was centrifuged for 15 min at 5000 rpm, and the supernatant was taken. Then, 1 ml of 5% EDTA solution and 0.5 ml of bromine water were added to the supernatant and mixed thoroughly. Formic acid was added drop by drop to remove excess brominated water. Then, 1 ml of 0.1% KI and 1 ml of leuco violet crystal solution were added. The pH of the solution was kept between 4.4–5.4 by using 0.5 M NaOH and formic acid. The mixture was diluted to 25 ml and left for 30 min for color development. Iodine contents were determined calorimetrically using a UV spectrophotometer (Model: LI-UV-7000) at 591 nm. For plant samples, a similar abovementioned procedure was adopted. All the chemicals used in the current study were analytical grade and purchased from Sigma Aldrich.

2.5. Nutritional analysis of plant leaves and shoots

A subsample from each plot was secured to analyze zinc (Zn), iron (Fe), phosphorous (P), calcium (Ca), magnesium (Mg), sodium (Na), and potassium (K) content. Shoot samples were washed rapidly with tap water and deionized water and then dried at approximately 45°C in a forced-draft oven to constant weight. The dried grains were ground to fine flour using an agate mill (Pulverisette 9, Fritsch GmbH, Germany) and digested with $HNO_3 + H_2O_2$ in a microwave digester. Ca, Mg, Na, K, Fe, and Zn were analyzed through atomic absorptive (AAS-), while P was determined using the blue method *via* UV spectrophotometer (Model: LI-UV-7000) at 700 nm.

2.6. Data analysis

The iodine concentrations in leaves and roots were calculated on a fresh weight basis. The transfer factor was calculated by using Equation (1):

$$TF = \frac{\text{Iodine concentration in shoot}\left(\mu g\ \text{kg}^{-1}\right)}{\text{Iodine concentration in soil}\left(\mu g\ \text{kg}^{-1}\right)} \quad (1).$$

Whereas the distribution constant was determined by using Equation (2):

$$DC = \frac{\text{Iodine concentration in shoot}\left(\mu g\ kg^{-1}\right)}{\text{Iodine concentration in root}\left(\mu g\ kg^{-1}\right)} \quad (2).$$

Effects of different treatments on the dependent iodine uptake, biomass production, and mineral composition were determined using two-way ANOVA at 0.05 level of least significant difference (LSD) SPSS. Figures were prepared using the ORIGIN software version 2020.

3. Results

3.1. Dry biomass

The application of amendments had a significant effect on both shoot and root biomass irrespective of the iodine treatments ($p < 0.05$) (Figure 1). The application of wood ash amended soil fertigated with KI treatment significantly increased the shoot ($40.3\,g\,m^{-2}$) and root biomass ($12.1\,g\,m^{-2}$) ($p < 0.05$). Soil amendments differed for coriander biomass (shoot) in the order of wood ash ($52.4\,g\,m^{-2}$) > charcoal ($50.8\,g\,m^{-2}$) > sawdust ($47.4\,g\,m^{-2}$) > gypsum ($45.1\,g\,m^{-2}$) > rice husk ($42.5\,g\,m^{-2}$). The coriander biomass was enhanced by 42% in the wood ash mixed soil supplemented with KI salt compared to the sole control.

3.2. Iodine uptake

Iodine accumulation in roots of coriander plants significantly varied ($p \leq 0.005$) among iodine sources (IS) ($F = 1688.38$, $p = 0.01$), and soil amendments (SA) ($F = 5589.72$, $p < 0.01$) (Table 3). A significantly higher I concentration was found in the roots of coriander plants that were grown in gypsum-amended soil fertigated with organic iodine ($240.4 \pm 1.29\,\mu g\,kg^{-1}$). However, the lowest iodine content in the roots was found in a plant grown in control settings (without amendment) fertigated with the organic iodine (OI) ($26.89 \pm 1.17\,\mu g\,kg^{-1}$). All plants grown in amended soils retained significantly higher iodine content irrespective of the source compared to respective controls. All the possible interactions between IS × SA had a significant effect ($F = 1196.34$, $p \leq 0.005$) on iodine uptake by the coriander grown in amended soil. The interactive effect of IS × SA revealed that the KI treatments significantly differed ($p \leq 0.05$) from OI regarding iodine uptake in the roots of the coriander plant (Figure 1). Maximum iodine was accumulated ($230\,\mu g\,kg^{-1}$) in roots of coriander grown in gypsum-amended soil fertigated with KI when compared with the control (Figure 2).

3.3. Iodine uptake by shoot

Coriander is usually utilized as a fresh shoot. Iodine accumulation in shoots of coriander plants significantly varied ($p \leq 0.005$) among I sources (IS) ($F = 1246.32$, $p = 0.01$) and soil amendments (SA) ($F = 5023.77$, $p < 0.01$) (Table 3). A significantly higher I concentration was found in coriander plant shoots grown in wood ash-amended soil fertigated with KI ($291.97 \pm 1.5\,\mu g\,kg^{-1}$). The combination IS × SA revealed a significant effect on the biofortification of iodine in the shoot ($F = 476.94$, $p < 0.01$) (Table 4). Iodine uptake by plants may be associated with soil properties that have modified the coriander's chemical composition and growth after applying soil amendments. The incorporation of wood ash showed a remarkable increase in iodine concentration in plants over control. Iodine concentration in the plant shoot was significantly higher with KI grown in wood ash amended soil ($291.97\,\mu g\,kg^{-1}$), while the lowest iodine was retained by a plant grown in gypsum amended soil fertigated irrespective of the source of iodine ($134.65\,\mu g\,kg^{-1}$) (Figure 2). The iodine retention in soil fertigated with KI differed as wood ash ($291.97\ \mu g\ kg^{-1}$) > rice hulk ($237.66\ \mu g\ kg^{-1}$) > sawdust ($217.10\ \mu g\ kg^{-1}$) > charcoal ($190.53\ \mu g\ kg^{-1}$) > gypsum ($134.28\ \mu g\ kg^{-1}$).

3.4. Transfer factor and distribution coefficient

Soil-to-coriander iodine transfer factor values (TF) are shown in Figure 3. The translocation factor of coriander plants added with KI

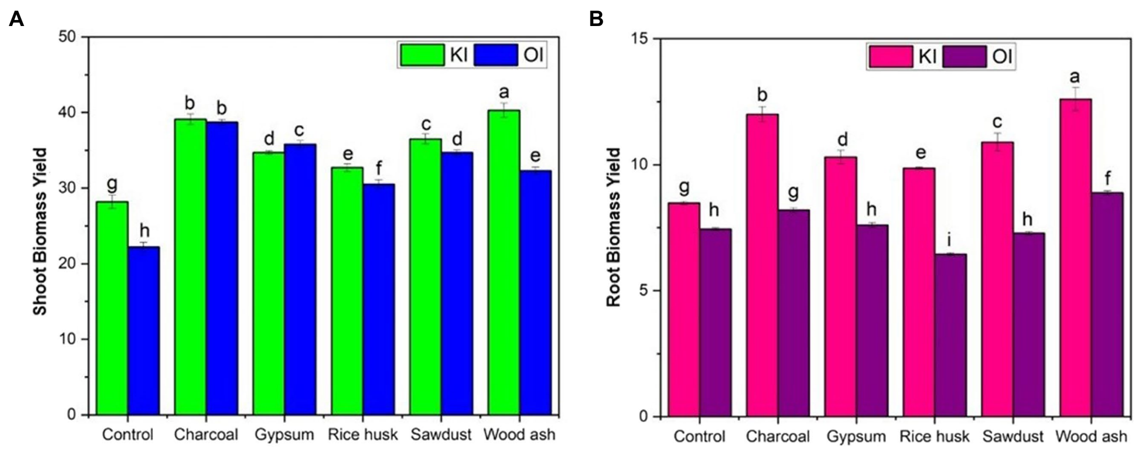

FIGURE 1
Shoot and root biomass yield of coriander plants after soil amendments as affected by **(A)** potassium iodide (KI) and **(B)** organic iodine (OI).

was significantly higher than the organic form of iodine. For plants treated with KI, the TF increased from 0.45 to 0.96 when the soil was amended with wood ash. The translocation factor declined with the application of organic iodine. A lower TF was found in the gypsum-amended soils either supplemented with inorganic or organic iodine. These values were slightly lowered in plants added with organic iodine. The distribution coefficient (DC) values of iodine between the shoot and root are given in Figure 3. The DC values for plants treated with KI increased significantly compared to the control. Wood ash application gave higher DC values (3.51, $p < 0.001$); while for plants treated with organic iodine, the sawdust treatment exhibited a higher DC value (1.56, $p < 0.01$) than the control. The effects of iodine sources, soil amendment, and their interaction significantly enhanced the concentration of iodine in coriander plants.

TABLE 3 Effect of different iodine sources (IS) and soil amendments (SA) on iodine uptake in roots of coriander crop.

Factors	Iodine uptake in the root (Mean±SE)
Iodine sources (IS)	
Potassium iodide	167.77 ± 2.29 a
Organic iodine	136.82 ± 3.37 b
Soil amendments (SA)	
Control	27.29 ± 1.16 f
Wood Ash	172.31 ± 2.24 c
Charcoal	143.03 ± 2.87 e
Gypsum	230.54 ± 3.11 a
Rice Husk	149.10 ± 1.97 d
Sawdust	191.52 ± 1.56 b
LSD (IS)	1.55
LSD (SA)	2.69
LSD (IS × SA)	3.8
F-value (IS)	1688.38**
F-value (SA)	5589.72**
F-value (IS × SA)	1196.34**

Means in similar columns with distinct lower case alphabets are significantly different at $p \leq 0.05$; **Significant at $p \leq 0.01$.

3.5. Nutrient concentrations

Soil amendment increased shoot concentrations of essential nutrients, irrespective of treatments (Table 5). The concentrations of nutrients in the coriander shoot varied in the order of K > P > Mg > Ca > Na > Fe > Zn. K concentrations in coriander shoots supplemented with KI increased by 9.2, 18.9, 28.6, 111.8, and 55.2% in the sawdust, rice husk, charcoal, wood ash, and gypsum treatments, respectively. Ca concentrations in coriander shoots increased by 30.2, 27.6, 14.5, 34.8, and 63.2% in the sawdust, rice husk, charcoal, wood ash, and gypsum treatments, respectively. Sawdust could not enhance Ca in coriander plants. Compared with the control, the amendment application improved P concentrations in coriander from 20.1 mg kg^{-1} (control) to 29.3 mg kg^{-1} in rice husk, 23.6 mg kg^{-1} in wood ash, and 20.3 mg kg^{-1} in gypsum treatment. Organic sources of iodine enhanced the concentration of nutrients in coriander plants. Gypsum treatment could not enhance the concentration of P in the coriander shoot. Changes in the mineral composition of coriander plants could be associated with the physicochemical variations that occurred within the soil due to the soil amendment.

4. Discussion and conclusion

Although some studies have demonstrated that different iodine types have different absorption or translocation rates by plants (Zhu et al., 2003), there is a lack of any concrete proof of a connection between soil solution iodine and plant uptake. The current study's findings showed that iodine uptake by coriander was closely connected to varied iodine levels in the soil. KI had a considerably higher capacity for iodine enrichment in coriander plants than OI. Iodine availability in soils, primarily controlled by adsorption–desorption processes, is a prerequisite for iodine uptake by plants

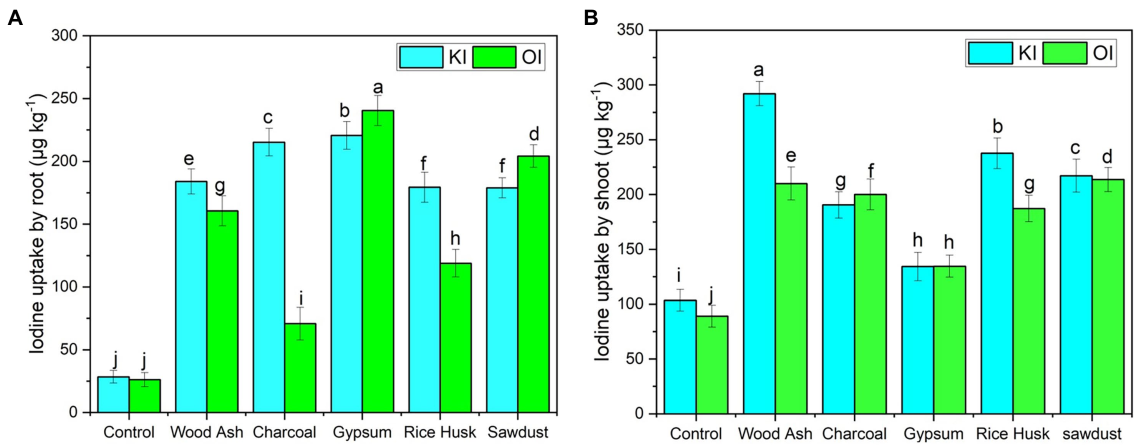

FIGURE 2
Iodine concentrations in the shoots and roots of coriander plants after soil amendments as affected by **(A)** potassium iodide (KI) and **(B)** organic iodine (OI).

growing in soils. The results showed that OI has a significantly higher affinity for the soil utilized in this experiment than KI; hence its availability in soils was lower than iodide. Therefore, the difference in iodine concentrations in soil solution, which is immediately available for root uptake, might account for the difference in iodine uptake by coriander plants supplied with KI and OI. Low iodine concentrations in soil solutions after OI treatments may be caused by significant iodine volatilization. According to Fuge (1996), the global iodine cycle and the transfer of iodine to the biosphere are significantly influenced by the volatilization of iodine from soils. Iodine in the soil was discovered to be volatilized from the soil–plant system into the atmosphere as organic iodine (Muramatsu et al., 1995; Kumar and Hemantaranjan, 2017). Organoiodides, volatile iodine molecules, can escape terrestrial ecosystems, including peat bogs and rice fields (Redeker et al., 2000; Dimmer et al., 2001), and the concentrations of iodide in soils eventually determine how much volatile organoiodides may be produced from soil (Keppler et al., 2003).

TABLE 4 Effect of different iodine sources (IS) and soil amendments (SA) on iodine uptake in shoots of coriander crop.

Factors	Iodine uptake in the shoot (Mean±SE)
Iodine sources (IS)	
Potassium iodide	195.85 ± 8.56 a
Organic Iodine	172.44 ± 9.23 b
Soil amendments (SA)	
Control	96.23 ± 4.78 f
Wood Ash	250.99 ± 5,71 a
Charcoal	195.31 ± 4.89 d
Gypsum	134.47 ± 7.89 e
Rice Husk	212.44 ± 4.65 c
Sawdust	215.41 ± 7.56 b
LSD (IS)	1.36
LSD (SA)	2.37
LSD (IS × SA)	3.35
F-value (IS)	1246.32**
F-value (SA)	5023.77**
F-value (IS × SA)	476.94**

Means in similar columns with distinct lower case alphabets are significantly different at $p \leq 0.05$; **Significant at $p \leq 0.01$.

Soil amendment significantly affected the plant biomass and nutritional value. The application of charcoal and wood ash produced higher biomass than other soil amendments. This might have resulted from adding soil amendments that affected soil properties and crop growth by improving soil pH, organic matter, and several nutrient concentrations, as well as crop emergence and yield relative to control treatment (Mohiuddin et al., 2019a). Wood ash, charcoal, and rice husk increased the iodine concentration in both root and shoot. The TF and DC of iodine between shoot and root were relatively higher for plants treated with KI. Similarly, wood ash showed a higher DC ratio than other soil amendments. The lower levels of iodine in the growth environment (0.02–0.2 mg kg^{-1}) benefit several plant species (Zhu et al., 2003).

The addition of soil amendment significantly enhanced the biomass of plants. This could be due to the provision of essential nutrients to plants. Phytofortification of iodine was higher with the addition of an iodide form of iodine compared to the organic source. The higher iodine accumulation in plant tissue with KI was also reported by (Zhu et al., 2003; Voogt et al., 2010; Humphrey et al., 2019). Mohiuddin et al. (2019b) reported enhanced bioaccumulation of iodine after adding different soil amendments. Applying organic amendments retained more iodine in the soil and reduced its losses *via* leaching.

Previous reports have shown that iodine was not phloem-mobile; therefore, iodine accumulation in grains was lower (Mackowiak and Grossl, 1999). For leafy vegetables, iodine accumulation in the edible parts is dependent mainly on the xylem transport. Therefore, one of the goals of the present study was to demonstrate whether iodine can be accumulated in coriander shoots for supplementary iodine nutrition. This study showed a

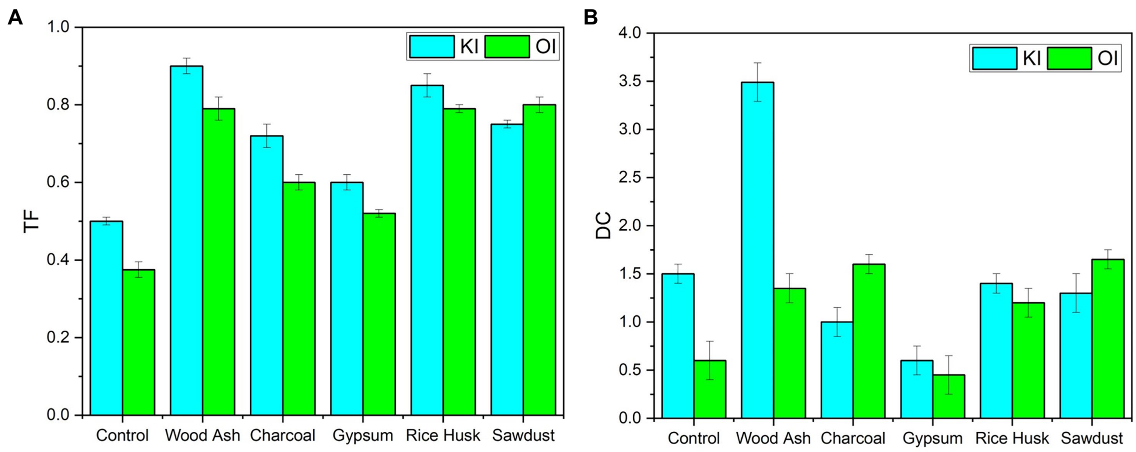

FIGURE 3
Iodine transfer factors (TF) and distribution coefficients (DC) of coriander plants after soil amendments as affected by **(A)** potassium iodide (KI) and **(B)** organic iodine (OI).

TABLE 5 Nutrient composition in coriander shoots as affected by soil amendments.

Source	Amendment	Ca	Mg	Na	K	Fe	Zn	P
	Control	15.2	17.2	14.2	38.0	2.6	0.8	20.1
Inorganic Iodine	Sawdust	19.8	22.4	16.2	41.5	2.8	0.9	22.8
	Rice husk	19.4	21.9	19.1	45.2	2.8	0.9	29.3
	Charcoal	17.4	19.7	16.0	48.9	2.7	0.8	20.0
	Wood ash	20.5	26.2	18.8	80.5	2.8	0.9	23.6
	Gypsum	24.8	16.7	13.6	59.1	0.6	0.7	20.3
Organic Iodine	Sawdust	15.6	17.7	14.4	52.8	2.6	0.7	17.0
	Rice husk	19.2	21.8	17.7	62.5	2.8	0.9	22.1
	Charcoal	22.8	25.8	21.0	53.3	2.6	1.1	16.7
	Wood ash	18.9	21.4	17.4	69.5	2.8	0.9	21.8
	Gypsum	21.5	21.0	17.1	66.6	1.7	0.8	21.3
	LSD (0.05)	1.2	1.4	0.9	2.7	0.4	0.5	1.8

higher iodine TF than reported for rice crops (Mackowiak and Grossl, 1999) and wheat grains (Shinonaga et al., 2001). It was observed that plants grown in gypsum-amended soil accumulated a lower amount of iodine content in plant shoots when compared with other soil amendments. Applying gypsum resulted in higher Ca content, lower iodine retention in soil (Fuge, 2013), and lesser iodine bioavailability (Hu et al., 2012). Ca-containing amendment increased soil pH and CEC and affected the soil's ability for iodine retention at higher pH (Fuge, 2013). Instead, the organic amendments enhanced the soil's organic carbon, which substantially enhanced the soil's capacity to retain iodine contents (Johnson, 2003). The wood ash amendment showed an improvement in iodine bioavailability from the soil.

The amount of iodine accumulation in the shoots was higher than in the roots of coriander plants. Soil properties such as cation exchange capacity, organic matter, and clay minerals have affected iodine adsorption and bioavailability (Yoshida et al., 1992). Changes in soil properties due to the soil amendment could be attributed to the enhanced mineral contents in coriander plants. Mohiuddin et al. (2021) reported the relationship of iodine with soil properties and predicted the substantial effects on the extractability of iodine contents from soils. This study confirms that the availability of iodine to coriander can be improved using a suitable soil amendment. Coriander edible shoots and leaves can absorb and store iodine when exogenous iodine fertilizer is used. After using the exogenous iodine fertilizers, KI, and seaweed composite, a clear difference was shown in the amount of iodine that plants could absorb.

It is concluded that the KI-fertigated soils accumulate higher iodine than the OI-fertigated soils. Soil amended with wood ash increased the iodine concentration in plant tissues. Higher values of TF and DC were recorded for plants treated with KI. Plants absorb more iodine from KI than from the seaweed composite. Favorable iodine addition can speed up the development of vegetables; however, too much iodine might be detrimental to them. Therefore, a suitable soil amendment can enhance iodine availability in soil. It is necessary to investigate iodine accumulation in vegetables under different soil amendments concerning soil attributes under field conditions.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Author contributions

YT and AmF: conceptualization. YT, MM, and AmF: methodology. MM and AsF: fieldwork and chemical analysis. YT, AA, and NG: data analysis. YT and MM: writing – original draft preparation. AA, AH, and MI: validation and writing – review and editing. AA: supervision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

MM was employed by Environmental Management Consultant (EMC) Pvt Ltd, Pakistan.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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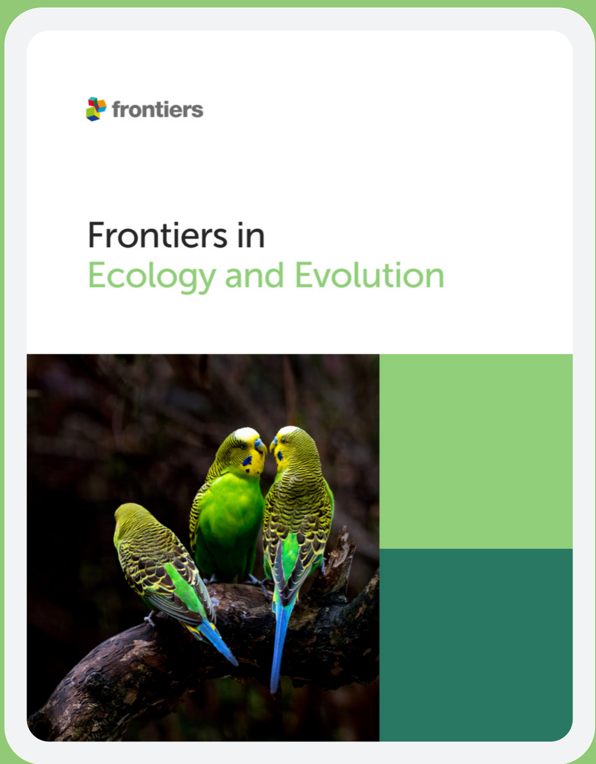

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