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Temporal Cumulative Effects on Soil Microbial Communities and Multifunctionality in Desert Oasis Expansion Areas: A Case Study of Zhangye Oasis (Postprint)

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Abstract

Abstract: Over the past 50 years, rapid population growth in arid northwestern China has driven continuous expansion of croplands and forestlands at desert-oasis ecotones into desert areas. However, research on soil microbial community succession and multifunctionality variation patterns in these desert-oasis expansion zones remains limited. This study examined croplands reclaimed and Haloxylon ammodendron plantations established for 5, 10, 20, and 30 years in the Zhangye Oasis expansion area of the Hexi Corridor. We analyzed 0–20 cm soil physicochemical properties (nutrients, pH, electrical conductivity, water content), soil enzymes (carbon, nitrogen, and phosphorus cycling enzymes), and soil microorganisms (bacterial and fungal communities via 16S rRNA gene and ITS region amplicon sequencing) to elucidate evolution patterns in community composition, structure, diversity, and multifunctionality, and their potential relationships. Results showed that cropland soil multifunctionality over 30 years exhibited an initial increase followed by decrease, peaking at 10 years and declining significantly from 20 years ($P<0.05$). Conversely, H. ammodendron plantation multifunctionality increased with planting age, reaching maximum at 30 years. In croplands, fungal communities showed more pronounced changes than bacterial communities; multifunctionality was significantly negatively correlated with fungal pathogens ($r=-0.655$, $P<0.01$) and positively correlated with α-diversity ($r=0.508$, $P<0.05$) and network complexity ($r=0.645$, $P<0.05$). Principal component analysis identified fungal pathogens as the primary factor influencing cropland soil multifunctionality. In plantations, bacterial communities contributed more strongly to multifunctionality changes, with significant positive correlations between multifunctionality and α-diversity ($r=0.546$, $P<0.001$) and network complexity ($r=0.542$, $P<0.001$). Bacterial α-diversity and structural complexity were key factors for plantation soil multifunctionality. These findings provide data support and scientific basis for soil health management in desert-oasis expansion zones.

Full Text

Preamble

Cumulative effects of time on soil microbial community and multifunctionality in a desert oasis expansion area: A case study of the Zhangye oasis

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Abstract

Over the past 50 years, rapid population growth in the arid regions of Northwest China has driven continuous expansion of farmland and shrubland into desert areas at desert-oasis margins. However, research on soil microbial community succession and multifunctionality changes in these desert oasis expansion zones remains limited. This study investigated newly cultivated farmlands and *Haloxylon ammodendron* plantations in the Zhangye oasis expansion area of the Hexi Corridor. We analyzed soil physicochemical properties (nutrients, pH, electrical conductivity, moisture), soil enzymes (carbon, nitrogen, and phosphorus cycling enzymes), and soil microorganisms (bacteria and fungi: 16S rRNA amplicon sequencing of the 0–20 cm soil layer) to elucidate the evolution of soil bacterial and fungal community composition, structure, diversity, and multifunctionality, as well as the potential relationships between soil multifunctionality and soil microorganisms.

The results revealed that over a 30-year reclamation period, farmland soil multifunctionality initially increased then decreased, peaking at 10 years and declining significantly thereafter ($P < 0.05$). In contrast, *H. ammodendron* plantation soil multifunctionality increased with plantation age, reaching its maximum at 30 years. In farmland, fungal communities exhibited more pronounced changes than bacterial communities, with soil multifunctionality showing a significant negative correlation with fungal pathogens ($r = -0.655$, $P < 0.01$) and significant positive correlations with α-diversity ($r = 0.508$, $P < 0.05$) and network complexity ($r = 0.645$, $P < 0.05$). Principal component analysis identified fungal pathogens as the primary factor influencing farmland soil multifunctionality. In shrubland, bacterial communities contributed more strongly to soil multifunctionality changes, with multifunctionality showing significant positive correlations with both α-diversity (r = 0.546, P < 0.001) and network complexity (r = 0.542, P < 0.001). Soil bacterial α-diversity and structural complexity emerged as key factors influencing shrubland soil multifunctionality. These findings provide data support and a scientific basis for soil health management in farmland and shrubland within desert oasis expansion zones.

Keywords: desert-oasis expansion zone; farmland; shrubland; bacteria; fungi; soil multifunctionality; time-dependent cumulative effect

Introduction

Oasification represents a human-dominated process of oasis agricultural expansion in arid desert regions, involving complex aboveground and belowground evolutionary processes. Multifunctionality serves as a comprehensive indicator for evaluating multiple ecosystem functions and properties, providing a multidimensional reflection of ecosystem services at specific timescales. In recent decades, rapid population growth and economic development in the inland arid regions of Northwest China have driven the continuous conversion of natural grasslands and fixed sand dunes into farmland and shrubland at desert-oasis margins, with the desert-oasis expansion zone extending further into desert areas. Agricultural and ecological development in these regions primarily includes oasis irrigation agriculture, shelterbelt system construction, and ecological restoration around farmland. Establishing ecological buffer zones around artificial oases is particularly crucial for maintaining stable oasis agriculture. However, excessive agricultural expansion often alters soil and eco-hydrological environments and affects the distribution and diversity of soil microorganisms, plants, and animals through ecological spillover effects, thereby impacting ecosystem functions and stability.

Soil bacteria and fungi are essential participants in organic matter decomposition and nutrient cycling, playing critical roles in maintaining soil multifunctionality. For instance, certain soil bacteria maintain soil organic matter and nutrient cycles through phosphorus solubilization or nitrogen fixation. Conversely, research has shown that soil pathogens increase significantly during the later stages of farmland and shrubland development, negatively affecting host plant health and productivity. However, the functions of soil microorganisms differ substantially across ecosystems. During grassland restoration, bacterial rather than fungal diversity controls soil ecosystem functions, whereas in forest ecosystems, the roles of bacteria and fungi change. In desert-oasis expansion areas where multiple landscapes coexist and land use conversions are frequent, soil multifunctionality exhibits considerable uncertainty across different time scales. In the Manas River Basin of Xinjiang, oasis farmland soil quality peaked at 10 years after reclamation, while in the Sangong River Basin, newly reclaimed farmland soil conditions first improved then deteriorated over a 30-year period. Nevertheless, research on soil microbial community evolution and the relationship between soil microorganisms and multifunctionality under different land use types (e.g., farmland and shrubland) in desert-oasis edge expansion zones remains scarce.

The Zhangye oasis, the largest artificial oasis in the Hexi Corridor, has seen many fixed sand dunes at its margins converted to farmland since the 1980s, while *H. ammodendron* plantations have been established as artificial sand-fixing vegetation around farmland. Farmlands cultivated at different times and *H. ammodendron* plantations of varying ages represent the main land conversion patterns and processes in this artificial oasis expansion zone. Therefore, this study selected newly cultivated farmlands (primarily growing seed corn, *Zea mays*) and *H. ammodendron* plantations in the Zhangye oasis expansion area as research objects. Through soil sampling and microbial analysis across different reclamation years, we aimed to reveal the changing patterns of soil multifunctionality and microbial community evolution, as well as the potential linkages between multifunctionality and microbial communities. The results provide data support and a scientific basis for the construction and soil health management of farmland and shrubland in desert oasis expansion zones.

1.1 Study Area and Soil Sampling

The study area is located near the Linze Inland River Basin Comprehensive Research Station in Pingchuan Town, Linze County, at the northern edge of the Zhangye oasis, where the Badain Jaran Desert meets the Linze oasis of Gansu Province (39°09 -39°21 N, 100°02 -100°21 E). The region has a temperate desert continental climate with mean annual precipitation of 117.1 mm (concentrated in June–September), mean annual temperature of 7.6°C, annual sunshine hours exceeding 3000 h, mean annual evaporation of 2337.6 mm, and a frost-free period of approximately 105 days. The average wind speed is $3.2\ \mathrm{m \cdot s^{-1}}$, with prevailing northwesterly winds. Soil types are primarily aeolian sandy soil and gray-brown desert soil, with coarse texture and low fertility.

Farmlands mainly cultivate seed corn. Old farmlands are located within the oasis interior with long cultivation histories, while newly cultivated farmlands were created by mixing aeolian sandy soil from artificially fixed sand areas with old farmland soil at the oasis edge, then amending with organic fertilizer (composted cattle manure). These newly cultivated farmlands were developed sequentially from 2005 to 2020, forming a long-term chronosequence. Both old and new farmlands differ in their initial soil conditions and management practices (irrigation and fertilization details in). *H. ammodendron* seedlings were planted in rows 2–3 m apart with 0.5–1 m spacing within rows, oriented perpendicular to the main wind direction. Seedlings were irrigated 1–2 times annually during the first 1–2 years, with no irrigation after establishment. The northern edge of the oasis has extensive, long-established artificial sand-fixing vegetation, with rain-fed *H. ammodendron* plantations of different ages (individual characteristics in).

We established short-, medium-, and long-term reclamation and planting gradients for farmland and *H. ammodendron* shrubland. Specific sampling sites for different reclamation years are shown in [Figure 1: see original paper]. Soil samples (0–20 cm) were collected from four reclamation-year farmlands and four plantation-age shrublands using a nested sampling method. At each site, we selected three plots (25 m × 25 m) spaced >500 m apart, and within each plot randomly selected three plants (randomly positioned for each plot). After removing surface litter, we collected rhizosphere soil from 0–20 cm depth around each plant. Soils from the three plots were mixed to form one composite sample per site, then divided into two subsamples. One subsample was stored at -20°C for DNA extraction and enzyme activity assays, while the other was air-dried for physicochemical analysis. Soil measurements included β-glucosidase (nitrophenol colorimetry), urease (sodium phenolate colorimetry), alkaline phosphatase (disodium phenyl phosphate colorimetry), soil organic carbon (potassium dichromate oxidation-external heating method), total nitrogen (Kjeldahl method using UDK140 analyzer, Italy), total phosphorus, pH and electrical conductivity (1:5 soil-water extract measured with Multiline F/SET-3 analyzer, Germany), and soil moisture (oven-drying method).

1.3 Soil Multifunctionality Calculation

Soil multifunctionality was calculated based on 15 indicators: 8 soil physicochemical properties and enzyme activities (moisture, salinity, organic carbon, total nitrogen, total phosphorus, β-glucosidase, alkaline phosphatase, and urease), 2 beneficial microbial groups (relative abundance of saprotrophic and symbiotrophic fungi at genus level, reflecting soil fertility and ecosystem stability), and 5 microbial diversity indices (α-diversity indices for bacteria and fungi, representing species richness and diversity). Before calculation, all functional indicators were standardized using min-max normalization: $(xi - mini)/(maxi - mini)$, where xi is the measured value, and $mini$ and $maxi$ are the minimum and maximum values across all plots. The soil multifunctionality index was calculated as the average of all standardized values: $MF = (1/K)\sum[g(xi)]$, where K is the number of measured functions and g is the standardization function, yielding MF values between 0 and 1.

1.4 Network Complexity Analysis

Microbial network complexity serves as an important indicator of ecosystem health and function. We calculated network complexity using six standardized topological parameters (average degree, average path length, diameter, density, clustering coefficient, and modularity), with equal weighting for each. Average degree reflects overall network structure and information transfer capacity. Average path length, diameter, density, and clustering coefficient indicate network compactness, connectivity, and communication efficiency. Modularity represents the degree of module partitioning, with values typically between 0.4–0.7 indicating significant clustering. Higher values for average degree, density, clustering coefficient, and modularity, combined with lower average path length and diameter, indicate greater network complexity. For parameters where larger values indicate sparser networks (average path length and diameter), we used their reciprocals (X^{-1}) in calculations. Network complexity was calculated as: $NC = (1/K)\sum[g(xi)]$, where K is the number of topological parameters and g is the standardization function, yielding NC values between 0 and 1.

Results

2.1 Soil Multifunctionality, Physicochemical Properties, and Enzyme Activity

Farmland soil multifunctionality showed a parabolic trend over 30 years of reclamation, peaking at 10 years and decreasing by 53.49% at 20–30 years compared to the 10-year peak. Soil total phosphorus and salinity increased with reclamation age, while pH reached its maximum at 5 years and minimum at 20 years. Alkaline phosphatase activity increased with reclamation age ().

Shrubland soil multifunctionality increased continuously with plantation age, rising by 81.58% at 30 years compared to 5 years. Soil nutrients (organic carbon, total nitrogen, total phosphorus) increased with plantation age. Soil pH and salinity first decreased then increased, while soil moisture first increased then decreased, with 10-year plantations showing higher moisture than other ages. Urease activity showed an increasing trend, alkaline phosphatase peaked at 10 years, and β-glucosidase showed no significant changes ().

2.2 Soil Microbial Community Composition

2.2.1 Dominant Microbial Phyla Farmland bacterial communities were dominated by *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Acidobacteria*, while fungal communities were dominated by *Ascomycota*, *Mortierellomycota*, and *Basidiomycota* (). Shrubland bacterial communities were dominated by *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Gemmatimonadetes*, and *Acidobacteria*, with *Actinobacteria* and *Gemmatimonadetes* showing significant differences across plantation ages. Shrubland fungal communities were dominated by *Ascomycota* and *Basidiomycota*, with *Ascomycota* abundance first increasing then decreasing, and *Basidiomycota* showing significantly higher abundance at 30 years ().

2.2.2 Dominant Microbial Genera and Their Changes Farmland soil bacterial and fungal community compositions differed significantly across reclamation years ($P < 0.05$). Bacterial communities were dominated by *Acinetobacter*, *Azospirillum*, and *Azoarcus* at 5 years; *Acinetobacter*, *Nitrosomonas*, and *Diaphorobacter* at 10 years; *Acinetobacter* and *Azospirillum* at 20 years; and *Acinetobacter* at 30 years. *Acinetobacter* abundance increased significantly, while *Azospirillum* decreased significantly at 30 years. Other abundant genera included uncultured members of *IMCC26134*, *Sphingomonas*, *Longimicrobiaceae*, *Subgroup_6*, *Sphingomonadaceae*, and *Gemmatimonadaceae*.

Farmland fungal communities at 5 years were dominated by *Mortierella* and *Alternaria*; at 10 years by *Mortierella*, *Geopora*, *Pseudeurotium*, *Agaricus*, *Neocamarosporium*, and the pathogen *Lectera*; and at 30 years primarily by *Acinetobacter*. Pathogen abundance increased significantly at 30 years.

Shrubland bacterial community composition showed no significant differences across plantation ages, while fungal community composition differed significantly ($P < 0.05$). Bacterial communities were dominated by uncultured bacteria, while fungal communities showed distinct successional patterns.

2.2.3 Functional Groups and Their Relationship with Multifunctionality

Farmland bacterial (phosphate-solubilizing bacteria) and fungal (pathogens) functional groups showed significant changes across the chronosequence. Phosphate-solubilizing bacteria, dominated by *Acinetobacter*, *Bdellovibrio*, *Rhodococcus*, and *Chryseobacterium*, were significantly more abundant at 20 years. Their abundance was negatively correlated with multifunctionality but positively correlated with soil salinity and alkaline phosphatase. Fungal pathogens, dominated by *Lectera*, *Neocamarosporium*, and *Monosporascus*, increased significantly at 30 years and were negatively correlated with multifunctionality ([Figure 3: see original paper]).

Shrubland bacterial (nitrogen-fixing and aerobic bacteria) and fungal (saprotrophs) functional groups decreased significantly with plantation age and were negatively correlated with multifunctionality. Symbiotrophic fungi increased significantly and were positively correlated with multifunctionality. Nitrogen-fixing bacteria, dominated by *Sphingomonas*, *Arthrobacter*, *Paracoccus*, and *Altererythrobacter*, were less abundant at 30 years. Their abundance was positively correlated with soil salinity and negatively correlated with β-glucosidase. Fungal pathogens were positively correlated with organic carbon, total phosphorus, and alkaline phosphatase ([Figure 3: see original paper]).

2.3 Soil Microbial Diversity

2.3.1 Alpha Diversity and Its Relationship with Multifunctionality

Farmland fungal alpha diversity differed significantly across reclamation years ($P < 0.05$), with Chao1 and Shannon indices decreasing significantly at 30 years. Fungal Chao1 was negatively correlated with soil salinity and positively correlated with multifunctionality. Bacterial alpha diversity showed no significant changes ([Figure 4: see original paper]).

Shrubland bacterial alpha diversity differed significantly across plantation ages ($P < 0.05$), with Chao1, Shannon, and Simpson indices increasing significantly at 30 years. Bacterial Chao1 was positively correlated with organic carbon, total nitrogen, and urease, and negatively correlated with soil salinity. Bacterial alpha diversity was significantly positively correlated with multifunctionality ([Figure 5: see original paper]).

2.3.2 Beta Diversity Changes Farmland fungal beta diversity differed significantly across reclamation years ($R = 0.080$, $P < 0.05$), while bacterial beta diversity showed no significant differences ($R = 0.318$, $P > 0.05$). Both bacterial and fungal beta diversity in shrubland differed significantly across plantation ages, with clear community differentiation ($R = 0.318$, $P < 0.05$) ([Figure 6: see original paper]).

2.4 Relationship Between Microbial Co-occurrence Network Complexity and Multifunctionality

Farmland fungal network complexity showed a parabolic trend, peaking at 10 years and decreasing at 20–30 years, and was significantly positively correlated with ecosystem multifunctionality ($P < 0.05$). Average path length and network diameter increased at 20–30 years, while clustering coefficient and modularity decreased, indicating more fragmented network structures. Network complexity was significantly positively correlated with soil organic carbon (, [Figure 7: see original paper]).

Shrubland bacterial network complexity increased continuously, reaching its maximum at 30 years, and was extremely significantly positively correlated with multifunctionality ($P < 0.001$). Average path length and diameter decreased after 10 years, while density and clustering coefficient increased, indicating higher connectivity and structural compactness. Network complexity was significantly positively correlated with total phosphorus and β-glucosidase (, [Figure 7: see original paper]).

2.5 Principal Component Analysis of Multifunctionality

For farmland, two principal components were extracted (eigenvalue > 1), explaining 70.68% of the variance. PC1 (54.37% variance) was primarily associated with fungal diversity and network complexity. For shrubland, two principal components explained 85.06% of the variance. PC1 (69.73% variance) was associated with bacterial diversity and network complexity, while PC2 (19.33% variance) reflected additional microbial characteristics (,).

Discussion

3.1 Changes in Farmland and Shrubland Ecosystem Multifunctionality

The conversion of fixed sand dunes to farmland and *H. ammodendron* plantations at desert-oasis margins substantially alters soil and vegetation environments, leading to marked changes in soil microbial composition, structure, functional groups, and multifunctionality. We found that farmland multifunctionality decreased significantly after 10 years of reclamation, with fungal pathogens increasing significantly from 20 years onward and fungal alpha diversity decreasing. Previous studies indicate that long-term chemical fertilizer application in farmland depletes soil organic matter, reduces beneficial microbial habitats, and increases pathogen proliferation, thereby decreasing multifunctionality over time. Fungal communities showed more pronounced changes than bacterial communities, likely due to their greater sensitivity to soil environmental changes.

In contrast, shrubland multifunctionality peaked at 30 years, coinciding with maximum bacterial alpha diversity and network complexity. This demonstrates that in desert ecosystems, ecosystem multifunctionality and stability increase with vegetation restoration, with more mature communities exhibiting greater stability. Desert shrubs serve as important "ecosystem engineers" in arid sandy regions, and their survival is closely linked to symbiotic and saprotrophic fungal guilds.

3.2 Effects of Soil Bacterial and Fungal Functional Groups on Multifunctionality

Farmland multifunctionality decline was associated with increased fungal pathogens, which can cause nutrient and microbial community imbalances that affect community structure and ecosystem functioning. We found a significant negative correlation between multifunctionality and pathogen abundance. While high-nutrient soils typically harbor more pathogens, particularly phosphorus-related pathogens, our study confirmed that farmland soil total phosphorus was significantly positively correlated with pathogen abundance.

Shrubland multifunctionality was significantly correlated with fungal saprotrophs. Previous research shows that increasing soil nitrogen decreases saprotrophic diversity and abundance, while nutrient enrichment promotes rapid pathogen growth, intensifying competition between symbionts and pathogens and ultimately inhibiting saprotrophs. Our findings align with this pattern: as plantation age increased, *H. ammodendron* litter and root exudates accumulated, gradually restoring soil nutrients that provided a foundation for pathogen growth, which in turn suppressed saprotroph reproduction.

3.3 Soil Microbial Alpha Diversity and Multifunctionality

Farmland fungal alpha diversity explained 26.32% of ecosystem multifunctionality variation. Intensive fertilization at desert-oasis margins particularly reduces soil organic matter, and phosphorus addition exacerbates organic matter depletion and environmental imbalance. Concurrently, groundwater irrigation causes salt accumulation, significantly reducing fungal diversity. Our results showed significant correlations between farmland fungal alpha diversity, soil salinity, and multifunctionality. The reduction in organic carbon likely affects soil structure, aeration, nutrient availability, and pathogen abundance, further decreasing fungal alpha diversity and multifunctionality.

Shrubland multifunctionality changes could be explained by bacterial community co-occurrence network complexity. Vegetation restoration age is significantly positively correlated with bacterial network complexity, and soil organic matter content shows significant positive correlations with bacterial diversity. As *H. ammodendron* plantation age increased, aboveground litter and root metabolites accumulated, increasing soil organic matter and total nitrogen while enhancing microbial diversity. Urease activity was the primary factor affecting bacterial alpha diversity, with enhanced urease activity promoting bacterial diversity. In nutrient-rich shrubland soils, higher glucosidase activity corresponded to more complex microbial community networks and functions. Organic carbon and total phosphorus are crucial for microbial growth and reproduction, supporting greater bacterial diversity. Therefore, shrubland multifunctionality appears more dependent on bacterial activity and diversity than fungal communities.

3.4 Effects of Microbial Co-occurrence Network Complexity on Multifunctionality

Farmland multifunctionality decline could be explained by reduced fungal network complexity. Agricultural tillage decreases microbial network complexity, and multifunctionality is significantly negatively correlated with network complexity in some systems. We found that farmland fungal network complexity was significantly positively correlated with soil organic carbon, as organic carbon provides essential energy for fungal growth. Reduced organic carbon limits fungal populations and weakens inter-fungal interactions, decreasing community complexity.

Shrubland multifunctionality was strongly linked to bacterial network complexity. Soil nutrient enrichment provides necessary conditions for diverse heterotrophic microorganisms to coexist. In fertile shrubland soils, glucosidase activity is higher and microbial networks are more complex. Organic carbon and total phosphorus are vital for bacterial growth, and abundant organic carbon supports more bacterial species. Consequently, shrubland multifunctionality depends more on bacterial communities, which are far more abundant than fungi and thus exert greater influence.

Based on these analyses, we propose a conceptual framework for interactions among soil physicochemical properties, microbial communities, and ecosystem multifunctionality in desert-oasis expansion zones ([Figure 8: see original paper]). In farmland, fungal communities play the primary role, with pathogens and alpha diversity jointly driving multifunctionality changes. In shrubland, bacterial communities are more important, with multifunctionality positively correlated with bacterial alpha diversity and network complexity.

Conclusion

Farmland soil multifunctionality peaked at 10 years after reclamation then declined significantly, showing a negative correlation with fungal pathogen abundance and positive correlation with fungal alpha diversity. Fungal communities were more important than bacterial communities in farmland, with pathogen increase and alpha diversity decline jointly reducing multifunctionality. In contrast, *H. ammodendron* plantation soil multifunctionality peaked at 30 years and was significantly positively correlated with bacterial alpha diversity and network complexity, indicating that bacterial communities play a more important role in shrubland ecosystems.

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