

## Response of Soil Ciliate Communities to Ecological Restoration under Different Restoration Models: Postprint

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### Abstract

To investigate the response of soil ciliate communities to ecological restoration under different reforestation patterns and to assess restoration efficacy using their community characteristics, three different reforestation forest type sampling sites (spruce and sea buckthorn mixed forest A1, spruce forest A2, sea buckthorn forest B1) and two control cropland sites (wheat field A0, pea field B0) were selected as study sites in the reforestation area of Duoshi Township, Tianzhu Tibetan Autonomous County, Gansu Province from April 2014 to July 2015. The “non-flooded petri dish method”, in vivo observation method, and direct cultivation counting method were employed to investigate soil ciliate community characteristics, while associated soil environmental factors at each site were measured, and correlations among soil ciliate community characteristics, vegetation community parameters, and soil environmental factors under different restoration patterns were analyzed. A total of 125 soil ciliate species were identified, belonging to 9 classes, 19 orders, 29 families, and 34 genera. The results showed that significant differences existed in soil ciliate community structure characteristics between reforestation sites and control sites ( $P < 0.05$ ), with decreased species similarity among reforestation sites and increased community complexity. Species number, density, species diversity index, evenness index, and richness index of soil ciliates at reforestation sites all increased significantly ( $P < 0.05$ ), exhibiting the ranking  $A1 > B1 > A2 > B0 > A0$  among sites. The successional trend of dominant taxa at each site shifted from Colpodida in control sites to Hypotrichida in reforestation sites. Results from correlation analysis and redundancy analysis demonstrated that following cropland-to-forest conversion, organic matter, water content, and total nitrogen content were the most important factors influencing ciliate community structure stability, with significant differences in soil ciliate community composition among different forest

types, indicating that soil ciliate community structure can be used as an indicator for evaluating the ecological restoration efficacy of reforestation efforts.

## Full Text

## Preamble

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### Response of Soil Ciliate Community to Ecological Restoration Under Different Reforestation Patterns

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## Abstract

Rapid economic development worldwide has led to global environmental degradation, making the restoration of degraded ecosystems a critical focus of modern ecological research. In the 1990s, China initiated the key project of returning farmland to forest to achieve ecological restoration benefits, including environmental protection, soil erosion mitigation, flood threat reduction, and biodiversity enhancement. While studies on farmland-to-forest conversion have gradually increased, most have emphasized forest patterns, ecological benefits, vegetation, and soil environment, with less attention to soil microbial eukaryotes (protists). Ciliated protists play crucial roles in microbial food webs by controlling bacterial populations through predation, transferring carbon and energy through food chains, and releasing nutrients. Their rich species diversity, short growth cycles, rapid community evolution, and sensitivity to environmental changes make them ideal bioindicators of environmental quality. However, research on soil ciliates has primarily focused on classification, morphogenesis, and molecular information, with limited attention to community structure changes after farmland conversion and their use in evaluating restoration effectiveness.

This study investigated the response of soil ciliate communities to ecological restoration under three different forest types in Duoshi Township, Tianzhu Tibetan Autonomous County, Gansu Province: A1 (*Picea asperata*-*Hippophae rhamnoides* mixed forest), A2 (pure *Picea asperata*), and B1 (pure *Hippophae rhamnoides*). These forests were previously destroyed for agricultural crops. Soil samples were collected from each forest type and from two control croplands (A0: *Triticum aestivum*, B0: *Pisum sativum*). Ciliates were isolated using the non-flooded Petri dish method, characterized through *in vivo* light microscopy observation, and enumerated via direct-culture counting. Physical-chemical pa-

rameters were also recorded to relate ciliate community characteristics with vegetation and environmental factors under different restoration patterns.

A total of 125 ciliate species belonging to 9 classes, 19 orders, 29 families, and 34 genera were recorded. Significant differences in soil ciliate community structure ( $P < 0.05$ ) were found between restored forest soils and control soils. Species richness, density, diversity index, evenness index, and abundance index were all significantly higher ( $P < 0.05$ ) in restored forest soils than in control cultivars, decreasing in the order  $A1 > B1 > A2 > B0 > A0$ . The dominant ciliate group in control soils (order Colpodida) was replaced by order Sporadotrichida in restored forest soils. Correlation and redundancy analyses both showed that soil water content, organic matter, and total nitrogen were the main factors influencing ciliate community structure in restored forest soils.

**Keywords:** soil ciliate communities; vegetation restoration measures; ecological restoration; ecological response; redundancy analysis

## 1. Site Description

Duoshi Township is located in the northern part of Tianzhu Tibetan Autonomous County, Gansu Province, covering an area of 312.7 km<sup>2</sup> at an elevation of 2,526–3,261 m. Situated in the upper reaches of the Gulang River within the Shiyang River basin, the region features a continental arid-semi-humid climate with an average annual temperature of 4.5°C and precipitation of 450 mm. The terrain is dominated by mid-mountain landforms with some loess hills. Vegetation types are complex, with main tree species including *Picea crassifolia* and *Populus davidiana*, and shrub species primarily consisting of *Spiraea alpina*. Soil types are mainly chestnut soil and gray-cinnamon soil.

Due to climate constraints and historical over-cutting and cultivation, watershed vegetation degraded severely, resulting in a fragile ecological environment with declining disaster resistance. Since the national farmland-to-forest program began in 1999, vegetation has gradually recovered, making this township a typical restoration area in Tianzhu County.

[Figure 1: see original paper] Schematic diagram of soil ciliate sampling sites

Five sampling sites were selected in the Chashugou and Hanquangou areas: three reforestation sites (A1: *Picea asperata*-*Hippophae rhamnoides* mixed forest, A2: pure *Picea asperata*, B1: pure *Hippophae rhamnoides*) and two control croplands (A0: wheat field, B0: pea field).

## 2. Sampling

At each site, a 100 m<sup>2</sup> sampling area was established. Soil samples were collected using the five-point sampling method with a soil corer. Each of the five points was further subsampled using the five-point method, resulting in each composite sample comprising 25 individual samples. Collected soils were placed in plastic

bags. During sampling, soil temperature, litter thickness, and shrub and herb coverage were measured. Soils from 0–5 cm depth were collected with a cutting ring for water content, porosity, and other physicochemical measurements.

### 3. Vegetation Survey Methods

Two types of quadrats were established: 10 m × 10 m for trees and 1 m × 1 m for herbs. Species composition and coverage of semi-shrubs were recorded using 1 m × 1 m quadrats. Ten small quadrats were used to 统计 plant frequency. Based on these measurements, various ecological parameters were calculated.

### 4. Soil Physicochemical Factor Determination

Soil pH was measured using the potentiometric method (soil:water = 1:2.5) with a TSS-85 soil humidity-acidity meter (Siping Electronic Technology Research Institute). Soil temperature was measured with a geothermometer. Water content was determined by the drying method. Available phosphorus was extracted with sodium bicarbonate, available potassium with ammonium acetate, and available nitrogen by UV spectrophotometry and indophenol blue colorimetry. Total nitrogen was measured by the Kjeldahl method, total potassium by flame photometry, and total phosphorus by sodium hydroxide fusion-molybdenum antimony colorimetry. Organic matter was determined by potassium dichromate volumetry. Electrical conductivity was measured by extraction, and porosity by the ring knife method.

### 5. Ciliate Research Methods

**Qualitative study:** The non-flooded Petri dish method [23] was used. Air-dried soil samples (50 g) were placed in 15 cm culture dishes, moistened with soil extract until fully infiltrated but not submerged, and cultured at 25°C for 20 days with continuous observation. Identification was performed using in vivo observation techniques [24–32].

**Quantitative study:** The direct culture counting method was used. Air-dried soil samples were cultured in 10 cm dishes at 25°C while maintaining constant water level until maximum count day (9, 10, 11 d). The culture dish was tilted at 1.1:1 ratio, the supernatant was completely removed and measured, and one drop was taken for counting under a microscope. Counts were converted to number of ciliates per gram of soil sample based on ciliate density in water (density per 1 mL water).

### 6. Statistical Analysis

Shannon-Wiener diversity index (H'), Pielou evenness index (J), Simpson dominance index (D), and Margalef richness index (d) were calculated. SPSS 19.0 was used for correlation analysis and independent samples t-test. Significance

was set at  $P < 0.05$ . Primer 6.0 was used for cluster analysis and multidimensional scaling (MDS). Canoco 4.5 was used for redundancy analysis (RDA). Arcview Gis 9.3 and Origin 9.0 were used for mapping.

## 2. Results and Analysis

### 2.1 Response of Soil Environmental Factors to Different Reforestation Patterns

Soil physicochemical data for all sites are shown in Table 1. Soil temperature and porosity showed little variation among sites with no significant differences. Soil pH values ranged from 6.93–6.97, indicating neutral soils with no significant differences among sites. However, soil water content in A1, A2, and B0 sites was significantly higher than in A0 and B0 ( $P < 0.05$ ). Available nitrogen and phosphorus in A0 and B0 sites were significantly higher than in reforestation sites (A1, A2, B1) due to artificial fertilization at the time of sampling ( $P < 0.05$ ). Available potassium in B0 was significantly higher than other sites ( $P < 0.05$ ). Total nitrogen, total phosphorus, and organic matter in reforestation sites were significantly higher than in control croplands ( $P < 0.05$ ).

Soil physicochemical factors at various sampling sites (Mean  $\pm$  SE)

Hierarchical cluster analysis of soil environmental factors showed that A0 and B0 sites clustered first, indicating greatest similarity between croplands. A1 and A2 sites clustered next, while B1 showed greater similarity with reforestation sites. This demonstrates that soil environment differed significantly between reforested and control cropland sites.

[Figure 2: see original paper] Dendrogram of cluster analysis on soil environmental factors at various sites

### 2.2 Vegetation Response to Different Reforestation Patterns

Based on the Engler classification system [33], plant surveys identified distinct vegetation distributions among sites. The A1 mixed forest showed the richest vegetation, followed by the B1 Hippophae forest, while A2 pure Picea forest had relatively simple vegetation. Dominant species included *Artemisia japonica* in mixed forest, *Roegneria kamoji* in Picea forest, and *Medicago sativa* in Hippophae forest. Species number, diversity, and coverage were lower at control sites than reforestation sites.

Parameters of plant community at various sampling sites

### 2.3 Ciliate Species Distribution and Community Composition

A total of 125 soil ciliate species were identified across all sites. Reforestation sites showed more complex community composition than cropland sites. At the order level, the dominant group shifted from Colpodida in cropland sites to Sporadotrichida in reforestation sites.

Community composition of soil ciliates at various sampling sites

[Figure 3: see original paper] Community composition of soil ciliates at various sampling sites

## 2.4 Dominant Species

Frequently occurring species were identified as dominants. *Colpoda edaphon* and *Cohnilembus fusiformis* were dominant at all sites. *Oxytricha fallax* was dominant at all reforestation sites. Control sites had relatively simple dominant species composition, while reforestation sites showed increased dominant species diversity and complexity, shifting from r-strategist Colpodida to K-strategist Sporadotrichida and Oxytrichida.

## 2.5 Cosmopolitan and Endemic Species

Species present at all sites were considered cosmopolitan, totaling 27 species (21.6% of total). Endemic species (present at only one site) increased significantly after reforestation. Control sites had 3 endemic species (2.4%), while reforestation sites had 22 endemic species (17.6%), including *Colpoda fastigata* at A0, *Urosoma karini* at B0, and *Cyclidium elongatum* at reforestation sites.

## 2.6 Soil Ciliate Community Parameters

Ciliate species number, diversity index, richness index, and evenness index were all higher at reforestation sites than control sites ( $P < 0.05$ ). Dominance index showed the opposite trend, being significantly lower at reforestation sites ( $P < 0.05$ ). The A1 site had the highest species number and diversity.

[Figure 4: see original paper] Characteristic parameters of soil ciliate community at various sites

## 2.7 Correlation Analysis Between Ciliate Community Parameters and Soil Environmental Factors

Correlation analysis showed significant relationships between ciliate parameters and soil factors. Species number was significantly positively correlated with water content, total nitrogen, and organic matter. Density was significantly positively correlated with water content and total nitrogen, and highly significantly with organic matter. Diversity index was significantly positively correlated with total nitrogen and negatively with total phosphorus. Richness index was significantly positively correlated with total nitrogen and organic matter.

Correlations between soil ciliate community characteristic parameters and soil environmental factors

## 2.8 Ordination of Ciliates and Soil Environmental Factors

Principal component analysis based on ciliates and soil environmental factors showed the first two axes explained 98.4% of variation. Axis 1 primarily reflected water content, porosity, total phosphorus, and organic matter. Axis 2 reflected available phosphorus and organic matter. RDA ordination showed ciliate density, diversity index, richness index, and evenness index were positively correlated with water content, organic matter, and total nitrogen, while dominance index was negatively correlated.

[Figure 5: see original paper] RDA ordination diagram of sampling sites and soil environmental factors

Correlation coefficients between soil environmental factors and ordination axes, eigenvalues, and ciliate species-soil correlations

## 3. Discussion

### 3.1 Plant Community Response to Different Reforestation Patterns

Vegetation restoration inevitably affects species diversity and community composition. Species diversity reflects community structure, degree of organization, and habitat differences. In this study, reforestation sites showed higher species number, diversity, evenness, and coverage than control sites, with mixed forests performing better than pure forests. Among pure forests, Hippophae rhamnoides showed higher diversity than Picea asperata, likely because shrub litter decomposes more easily, improving soil moisture retention and nutrient accumulation. These results align with previous studies showing that mixed forests enhance biodiversity more effectively than pure stands, suggesting that reforestation programs should consider species composition and planting patterns.

### 3.2 Soil Environmental Factors Response to Different Reforestation Patterns

Vegetation restoration significantly altered soil properties. Compared with control croplands, reforestation sites showed increases in soil water content (17.24%), organic matter (29.18%), and total nitrogen (27.17%). Available potassium was slightly higher in reforestation sites, though the control site showed elevated levels due to fertilization. These improvements in soil quality demonstrate the positive impact of reforestation on soil ecosystems.

### 3.3 Soil Ciliate Community Response to Different Reforestation Patterns

As soil environment improved through reforestation, ciliate community structure changed accordingly. Despite being microscopic single-celled eukaryotes, ciliates respond rapidly to environmental changes due to their high diversity and fast

growth rates, making them excellent indicators of soil ecosystem changes. Reforestation sites showed higher species richness, abundance, and diversity than croplands. The dominant group shifted from r-strategist Colpodida (widely distributed, short reproductive cycles) to K-strategist Sporadotrichida (complex physiology, longer cycles), indicating more stable environmental conditions. This successional trend from r- to K-strategists confirms that reforestation improves soil habitat quality.

### 3.4 Influence of Vegetation and Environmental Factors on Ciliate Communities

Post-reforestation soil recovery is reflected in changed environmental factors that subsequently affect ciliate community parameters. Correlation and ordination analyses identified organic matter content as the most influential factor, followed by total nitrogen and water content. Mixed forests supported more stable and complex ciliate communities than pure forests, indicating better restoration outcomes. This supports the promotion of mixed-species plantings in restoration programs.

## 4. Conclusion

Quantitative analysis of vegetation, soil physicochemical factors, and soil ciliates under different reforestation patterns revealed:

1. Reforestation significantly altered soil environment and ciliate community composition. Improved vegetation increased water content, organic matter, and total nitrogen, creating more favorable conditions for ciliates.
2. Mixed forests were more effective for ecological restoration and biodiversity enhancement than pure stands. Among pure forests, *Hippophae rhamnoides* supported higher vegetation diversity and more stable ciliate communities than *Picea asperata*.
3. Ciliate species number and density correlated with vegetation species number and coverage, while ciliate diversity, dominance, and richness correlated with vegetation diversity parameters. These relationships demonstrate that ciliate community parameters can indirectly reflect vegetation recovery status.
4. Soil ciliate communities effectively responded to ecological restoration through farmland conversion, providing a biological indicator for evaluating restoration success. This study supplements previous research by examining different reforestation patterns and provides systematic baseline data for future studies.

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