Distribution of Endophytic Fungi in Common Poaceae Plants and Their Influencing Factors in Pangquangou Nature Reserve: Postprint

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Abstract

Taking the dominant grasses under four different community types of forests and shrublands in Pangquangou Nature Reserve as the research object, this study investigated the endophytic fungal infection rates of different grasses and examined the relationships between endophyte-infected grass populations and soil physicochemical properties, soil enzyme activities, and soil microbial community structure in their native habitats. The results showed that the main factors affecting the infection rates of Festuca rubra, Carex durieui, and Avena fatua were soil moisture content and the carbon-to-nitrogen ratio, and that soil C, N, S, and soil moisture content were positively correlated with the endophytic fungal infection rate of Festuca rubra. Soil sucrase, urease, and acid phosphatase were significantly positively correlated with the infection rate of Festuca rubra, but the results for Carex durieui were opposite to those for Festuca rubra. Inocybaceae were the dominant fungi in the soil microbial communities of Larix principis-rupprechtii forests and Pinus tabuliformis forests, but the composition of dominant bacterial microbial communities differed under different endophyte-infected vegetation community types. Overall, the correlations between grass endophytic fungal infection rates and soil physicochemical properties differed among the four forest community types, and the magnitude of influence by ecological factors also varied. Endophytic fungal infection led to differences in soil microbial community structure among vegetation community types, with different compositions of dominant soil fungi and soil bacteria. This helps to further understand the effects of grass populations with different infection rates on soil microbial community structure and their diverse ecological functions in ecosystems under native habitat conditions.

Full Text

Preamble

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Distribution of Endophytic Fungi Associated with Common Grasses and Preliminary Assessment of Impact Factors in Pangquangou Nature Reserve

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Abstract

This study investigated the dominant grasses under four different forest and shrub community types in Pangquangou Nature Reserve to examine endophyte infection rates and their relationships with soil physicochemical properties, enzyme activities, and microbial community structure in native habitats. Our results showed that soil water content and carbon-to-nitrogen ratio (C/N) were the primary factors affecting infection rates in *Festuca rubra*, *Carex alexeenkoana*, and *Avena fatua*. Soil C, N, S, water content, invertase, urease, and acid phosphatase were positively correlated with the endophyte infection rate of *F. rubra*, though *C. alexeenkoana* showed opposite patterns. Inocybaceae was the dominant fungal family in the soil microbial communities of *Larix principis-rupprechtii* and *Pinus tabuliformis* forests, while the composition of dominant bacterial communities differed under various infected vegetation types. The correlation between grass endophyte infection rates and soil physicochemical properties varied among community types, as did the magnitude of influence from ecological factors. Endophyte infection altered soil microbial community structure, leading to different compositions of dominant soil fungi and bacteria across forest community types. These findings enhance our understanding of how different infection rates in native grass populations influence soil microbial community structure and their distinct ecological functions in ecosystems.

Keywords: fungal endophyte; endophyte infection rate; enzymatic activity; soil microbial community

Introduction

Fungal endophytes are fungi that live internally within healthy plants for a significant portion of their life cycle without causing apparent disease symptoms. In grasses, these endophytes are often specialized, systemic, and vertically transmitted. They are widely distributed across host plants, including conifers and algae, with particularly high prevalence in Poaceae. Globally, endophytes have been found in at least 80 genera of grasses. Most endophytic hyphae reside

in the apical meristematic tissues of stems and leaves, with higher densities in basal stem meristems than in leaf sheaths and blades. Studies have shown that endophyte abundance and distribution vary significantly across different growth stages of host plants.

Surveys of natural grassland ecosystems worldwide reveal substantial variation in infection rates. In Finland, infection rates in tall fescue range from 4% to 82%, while in Denmark, perennial ryegrass infection varies between 44% and 92%. In North Africa (Morocco, Tunisia) and Sardinia, tall fescue infection rates range from 57.5% to 89.3%. Vinton et al. found that populations of *Elymus canadensis* in North American prairies had infection rates of 4%–82%. In China, Nan Zhibiao et al. surveyed *Elymus dahuricus* across 8 regions, finding infection rates of 4.4%–100% in seeds. Ahholm et al. demonstrated that both environment and host genotype interact to determine infection rates when environmental conditions change.

While many studies indicate mutualistic relationships between endophytes and cultivated grasses, symbioses with native grasses range from antagonistic to mutualistic. Endophyte effects extend beyond host plants to influence soil microorganisms in the surrounding habitat. Soil microbes drive ecosystem processes including material cycling, energy flow, organic matter decomposition, and nutrient transformation, serving as both nutrient reservoirs and sources of plant-available nutrients. They represent important indicators of soil fertility and nutrient bioavailability. Recent research has increasingly focused on how grass endophytes affect soil microbial community structure and function. For example, endophyte infection has been shown to increase bacterial, Gram-negative bacterial, and fungal phospholipid fatty acid (PLFA) contents in pot experiments with *Leymus chinensis*, while also increasing Gram-positive bacteria and actinomycete PLFAs in field plots. Casas et al. found that high levels of *Neotyphodium occultans* in ryegrass increased soil fungal activity and affected microbial metabolic diversity. However, most studies on native grass endophytes have focused on cultivated grasses or pot experiments, with few investigations examining their distribution in native habitats and impacts on soil microorganisms.

This study addresses this gap by examining endophyte distribution and infection rates in dominant grasses across different community types in Pangquangou Nature Reserve, investigating relationships with soil properties and enzyme activities, and exploring how endophytes influence soil microbial community structure to understand the distinct ecological functions of grass populations with varying infection rates in native ecosystems.

1. Study Materials

1.1 Study Site and Sampling

Pangquangou Nature Reserve is located in the middle section of the Lüliang Mountain range in northwestern Jiaocheng County and Fangshan County,

Shanxi Province (110°22 -37°55 N). The reserve was established for conservation of the first-grade state protection animal *Crossoptilon mantchuricum* and cold-temperate coniferous forests. It plays significant roles in water conservation, eco-tourism, and biodiversity maintenance. The region has an average annual temperature of 3–4°C, average annual precipitation of 800 mm, and relative humidity of 80%.

In May 2015, we selected four plots representing different community types: *Caragana jubata* shrubland, *Populus davidiana* forest, *Larix principis-rupprechtii* forest, and *Pinus tabuliformis* forest (elevation 1600–2831 m). In each plot, we collected dominant grasses including *Festuca rubra*, *Carex alexeenkoana*, *Avena fatua*, and *Poa annua*. Plant samples were stored in ice boxes for laboratory analysis. Soil samples were collected at 5–10 m intervals, with 3–4 samples per plot. One portion of soil was air-dried for physicochemical analysis, while another portion was mixed and stored at -20°C for high-throughput sequencing. Sampling location, time, soil type, and community information were recorded for each point .

1.2 Endophyte Detection

Endophytes in leaf sheaths were detected using aniline blue staining. Mature leaves were cut and the leaf sheath upper epidermis was removed with a scalpel and tweezers. The tissue was placed on a slide with aniline blue stain, heated over an alcohol lamp for 10–20 minutes, covered with a coverslip, and excess stain was removed. Samples were observed under an optical microscope; abundant dark blue hyphae indicated infection. Infection rate was calculated as the percentage of infected plants among all tested individuals of each grass species.

1.3 Soil Physicochemical Properties

Soil total carbon (C), nitrogen (N), and sulfur (S) were measured using an elemental analyzer (vario EL/MACRO cube, Elementar, Hanau, Germany). Soil bulk density was determined by the specific gravity bottle method, water content by the ring knife method, and porosity and particle size using a laser diffraction particle size analyzer (Mastersizer 3000, Malvern Co. Ltd, UK). Soil enzyme activities were measured as follows: urease activity by sodium hypochlorite colorimetry, catalase by potassium permanganate titration, invertase by phenol-sodium dinitrosalicylic acid colorimetry, and acid phosphatase by disodium phenyl phosphate colorimetry.

1.4 Soil Microbial Community Structure

Soil microbial community composition was analyzed by high-throughput sequencing. Genomic DNA was extracted using the OMEGA soil extraction kit and quantified with Qubit 2.0. For bacteria, the V3–V4 region was amplified using primers 341F (CCTACGGGNGGCWGCAG) and 805R (GACTGGAGTTCTTGGCACCCGAGAATTCCAGACTACHVGGGTATCTAATCC)

fused with Illumina MiSeq platform adapters. For fungi, the ITS1 region was amplified using primers ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS2-Rev (GCGTTCCTCATCGATGC). PCR products were sequenced by Sangon Biotech (Shanghai) Co., Ltd.

1.5 Data Analysis

Data were processed and plotted using Microsoft Excel. SPSS 19.0 was used for one-way and multi-way ANOVA, and CANOCO 4.5 for constrained ordination analysis.

2. Results

2.1 Endophyte Infection Rates in Dominant Grasses Across Communities

The endophyte infection rate of *Festuca rubra* in the *Caragana jubata* shrubland plot reached 100%, significantly higher than that of *Carex alexeenkoana* in the *Pinus tabuliformis* forest plot (30.9%). *Poa annua* showed infection rates of 30.9%–54.9% across plots, with no significant differences among the four community types. *Carex alexeenkoana* and *Avena fatua* also showed no significant differences in infection rates between plots, indicating that geographic population differences had little effect on infection rates for these species, though infection varied substantially among plant species [Figure 1: see original paper].

2.2 Effects of Soil Physicochemical Properties on Grass Endophyte Infection Rates

To determine how ecological factors influence infection rates and identify key factors for each grass species, we performed redundancy analysis (RDA) using CANOCO 5.0 on four soil physicochemical properties and four enzyme activities. The first ordination axis explained 39.3% of variation, with soil water content and C/N ratio being the main factors affecting infection rates in *F. rubra*, *C. alexeenkoana*, and *A. fatua*. The second axis explained 93.7% of variation, primarily related to total carbon. Correlations between soil properties, enzyme activities, and infection rates varied among grass species. Soil C, N, S, water content, invertase, urease, and acid phosphatase were positively correlated with *F. rubra* infection, while *C. alexeenkoana* showed opposite patterns. *Avena fatua* infection was positively correlated with C/N ratio and catalase, whereas *Poa annua* was only negatively correlated with soil carbon content [Figure 2: see original paper].

2.3 Effects of Different Infection Rates on Soil Microbial Community Structure

High-throughput sequencing revealed distinct bacterial community compositions at the family level across plots. In the *Larix principis-rupprechtii* for-

est, the dominant bacterial families were Chitinophagaceae (9.84%), Sphingomonadaceae (5.15%), Gemmatimonadaceae (3.68%), and Enterobacteriaceae (4.01%). In the *Pinus tabuliformis* forest, Planctomycetaceae (9.27%), Rhodocyclaceae (4.32%), and Sphingomonadaceae (4.12%) dominated, with greater variability in relative abundance. This indicates that bacterial community composition differed under different infected vegetation types.

At the fungal family level, Inocybaceae dominated both forest types, accounting for 37.67% of the fungal community in *P. tabuliformis* forest and 51.25% in *L. principis-rupprechtii* forest. Other abundant families included Thelephoraceae (19.87%), Hygrophoraceae (18.59%), and Cortinariaceae (10.52%) in *P. tabuliformis* forest, and Mortierellaceae (9.84%) in *L. principis-rupprechtii* forest [Figure 3: see original paper] [Figure 4: see original paper].

3. Discussion

3.1 Effects of Community Type on Dominant Grass Endophyte Infection Rates

Previous studies have reported high variability in endophyte infection rates among natural populations. For example, *Festuca rubra* infection by *Epichloë festucae* in Mediterranean grasslands ranges from 4% to 92%. Similarly, *Achnatherum sibiricum* populations show infection rates of 44%–92%, with some populations reaching 100%. In contrast, our study found no significant differences in infection rates for *C. alexeenkoana* and *A. fatua* between plots (30.9%–54.9%), suggesting that geographic population differences have limited influence on these species. However, infection rates varied substantially among plant species. According to Clay and Schardl, populations with intermediate infection levels are unstable, with rates that may increase or decrease depending on environmental selection pressures. Spatial and temporal variation, seasonal changes, humidity, and other plant groups can all influence endophyte distribution. From an ecosystem perspective, populations with different infection rates may have distinct ecological functions, representing both a condition for system maintenance and an outcome of ecosystem evolution.

3.2 Effects of Soil Properties and Enzyme Activities on Endophyte Infection Rates

Lewis et al. found that wild ryegrass infection levels correlated significantly with climatic factors, particularly evapotranspiration and water deficit, which accounted for 31% of total variation. Similarly, our results indicate that soil water content and C/N ratio are primary factors affecting infection rates in *F. rubra*, *C. alexeenkoana*, and *A. fatua*. The positive effects of endophytes may manifest during critical periods, such as extreme drought or rapid population decline, where the symbiont exhibits strong competitiveness. We also found positive correlations between soil C, N, S, water content and *F. rubra* infection rates, possibly because endophyte infection enhances soil C, N, and S accumu-

lation. However, *C. alexeenkoana* showed opposite results, while *P. annua* was only weakly affected by soil properties and enzyme activities. These differences suggest that distinct grass species have varying relationships with soil factors in native habitats.

3.3 Effects of Different Infection Rates on Soil Microbial Community Structure

Studies on endophyte effects on soil microbial communities have yielded inconsistent results, with some showing increased microbial biomass while others show reduced bacterial abundance. Rudgers and Clay found that endophyte symbiosis impacts communities more than individual populations. Our results demonstrate that while fungal community composition was similar between *L. principis-rupprechtii* and *P. tabuliformis* forests (both dominated by Inocybaceae), bacterial community composition differed significantly. This suggests endophyte infection may influence bacterial communities more than fungal communities. The significant positive correlation between *F. rubra* infection and soil invertase, urease, and acid phosphatase activities indicates that endophytes can alter soil enzyme activities, while the opposite patterns in *C. alexeenkoana* highlight species-specific responses.

The mechanisms by which endophytes alter soil microbial communities remain unclear. Possible explanations include: (1) endophytes modify the chemical composition of senescent leaves, affecting decomposition rates and consequently microbial communities; and (2) endophytes alter root exudate composition, changing soil physicochemical properties and microbial structure. Further research is needed to elucidate these pathways and their implications for ecosystem functioning in native grassland systems.

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Note: Figure translations are in progress. See original paper for figures.