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Original article

Tempo-spatial dynamics of arbuscular mycorrhizal fungi under clonal plant *Psammochloa villosa* Trin. Bor in Mu Us sandland

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ABSTRACT

Tempo-spatial dynamics of AM fungi within the rhizome system of *Psammochloa villosa* (Poaceae) were investigated in Mu Us sandland, northwest China. Soil samples in the annual and perennial ramet rhizospheres of *P. villosa* were collected in 2007. AM fungal percent colonization reached maximal values in the rainy season and spore number in the dry season. Spore number exhibited positive correlation with soil pH and available phosphorous (P) ($P < 0.01$), and negative correlation with available nitrogen (N) ($P < 0.05$). Vesicular, arbuscular, hyphal and total colonization were positively correlated with soil organic matter and available P ($P < 0.01$), and negatively correlated with available N ($P < 0.01$). Fourteen species of AM fungi in four genera were isolated. The same AM fungal taxa were found in the annual and perennial ramet rhizospheres, although the last ones had higher fungal colonization and spore number. A high Shannon-Weiner diversity index of AM fungi was observed. Spore number and species richness indicated that *Glomus* was the predominant AM fungi, especially the small-spored taxa. AM fungal dynamics under *P. villosa* are highly seasonal: different aged ramets and nutrient availability have effects on AM fungal development and abundance in Mu Us sandland.

1. Introduction

Arbuscular mycorrhizal (AM) fungi are a major component of the rhizosphere microflora in natural ecosystems. In some cases, AM fungi appear to be necessary for normal growth and survival of desert plants [9,10,22,42]. Mycorrhizal associations with plants can enhance the nutrient uptake by roots [23,34], alleviate drought stress [5,33], and accelerate plant establishment, growth and survival of seedlings [9,10]. Therefore, AM associations play an important role in the restoration and reestablishment of vegetation in fragile or degraded ecosystems [9,10,19], and in the maintenance of plant biodiversity and ecosystem functioning [14,43,44].

Clonal plants are propagated asexually by means of buds, tillerings, or shoots and represent the earliest stage of ecesis. Once established, propagules may form new independent units (ramets). However, ramets often stay connected to the parent for a time, resulting in a group [2]. Clonal species may have advantages over non-clonal species in the process of ecological restoration as they may be more adaptive to a hostile environment and have superior survival ability [20,31].

Clonality is a means of buffering microenvironmental heterogeneity to clonal plants [3]. AM fungi may play an important role in this process [38,39,41]. One mechanism by which environmental buffering within rhizome systems is obtained is through a division of labor among ramets, either due to the differential functioning of ramets in different resource environments [3] or due to changes in ramet function with age [21]. Since ramets differ in age and/or resource environment, this may result in a complex pattern of resource uptake and sharing that may cause similarly complex influences on AM fungi [46]. Thus, understanding how both clonal plant and their associated AM fungi develop will help to elucidate the ecological significance of this AM symbiosis, especially for arid environments.

The Mu Us sandland is the largest mobile sand dune system in the dry and nutrient-poor grassland of northwestern China, where, because of estrepement, denudation, over-grazing, mining disturbances, and excessive use of groundwater, many fix-sand dunes have degenerated into semi-fixed and mobile sand dunes, and desertification is increasing [11,24]. *Psammochloa villosa* (Trin.) Bor (Poaceae) is an important pioneer plant of the semi-fixed and mobile dunes in the desert, and plays an important role in fixing moving sand, and conserving biodiversity in Mu Us area [15,47]. Our objectives were to explore the tempo-spatial changes of AM fungi within the rhizome system of *P. villosa* in Mu Us sandland, and determine the effects of

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different aged ramets and edaphic factors on AM fungal distribution and abundance over a growing season.

2. Materials and methods

2.1. Site description

The study site (39°29′ N, 110°11′ E; 1308 m a.s.l.) was selected at Shilangmiao in Ordos Sandy Land Ecological Station of the Institute of Botany, the Chinese Academy of Sciences. This area is located in the southeast of the Mu Us sandland in Inner Mongolia, China (Fig. 1A). The climate is typical semi-arid type with remarkable seasonal and diurnal temperature variation. Annual mean precipitation is 345.2 mm with annual mean potential evaporation 2535 mm, most rainfall occurs during rainy season (July–September) and accounts for over 70% of annual precipitation. The soil is a nutrient deficient sandy soil. The landscape is characterized by mobile and semi-fixed sand dunes, and patchy vegetation dominated by psammophytic grasses, such as *P. villosa* [48]. The growing season is from late April to October.

2.2. Soil sampling

Six surveyed couples of 1 m^2 plots that included annual and perennial ramet types were designed along the axis of the rhizomes within the rhizome system of *P. villosa* randomly on semi-fixed dunes in Mu Us sandland in March 2007 (Fig. 1B). The distance between any couple of plots was at least 150 m. Soil samples (the rhizospheric soils) were collected in 1 for each type plot for 6 total replicates in May, July and October 2007, respectively. Surface soil (approximately 1–2 mm) was removed, and soil cores of 0–50 cm were divided into 5 sections, i.e. 0–10 cm, 10–20 cm, 20–30 cm, 30–40 cm and 40–50 cm. The collected soil and root samples were stored separately in self sealing polythene bags and placed in an insulated carrier for transport to the laboratory. Soil samples were air-dried and stored at 4 °C until analysis. The root samples were processed immediately.

2.3. Soil parameters

Soil samples, air-dried and sieved (2 mm mesh size), were analyzed for pH on 1:2.5 soil water suspension using a digital pH meter (PHS-3C, Shanghai Lida Instrument Factory). Soil organic matter was calculated from the percent organic carbon estimated by oxidization with dichromate in the presence of H_2SO_4 [32]; soil available N, using alkali hydrolysis diffusion method, and soil available P by chlorostannus-reduced molybdophosphoric blue color method by extraction with 0.5 M sodium bicarbonate for 30 min according to Olsen et al. [26,27].

2.4. Quantification of AM fungal colonization

Fresh roots were cut into 1 cm long pieces and processed by washing them free of soil. Then they were cleared with 10% (w/v) KOH and stained with 0.5% (w/v) acid fuchsin solution using the method described by Phillips and Hayman [30]. Fungal colonization was quantified by the glass slide method [17], in which 50 randomly chosen root segment units were examined at 100–400× magnification using a Nikon YS100 microscope for the presence of AM fungal structures. Five vision fields were examined in each root segment, resulting in 250 fields examined for each sample. A segment was counted as infected when arbuscules, vesicles, or hyphae were observed. Arbuscular, vesicular, hyphal and total colonization of AM fungi were expressed as the ratio of the number of colonized root segments to the total number of root segments examined.

2.5. Spore extraction

Spores of AM fungi were extracted from the soil by wet sieving and decanting, sucrose-gradient centrifugation [13]. All the spores

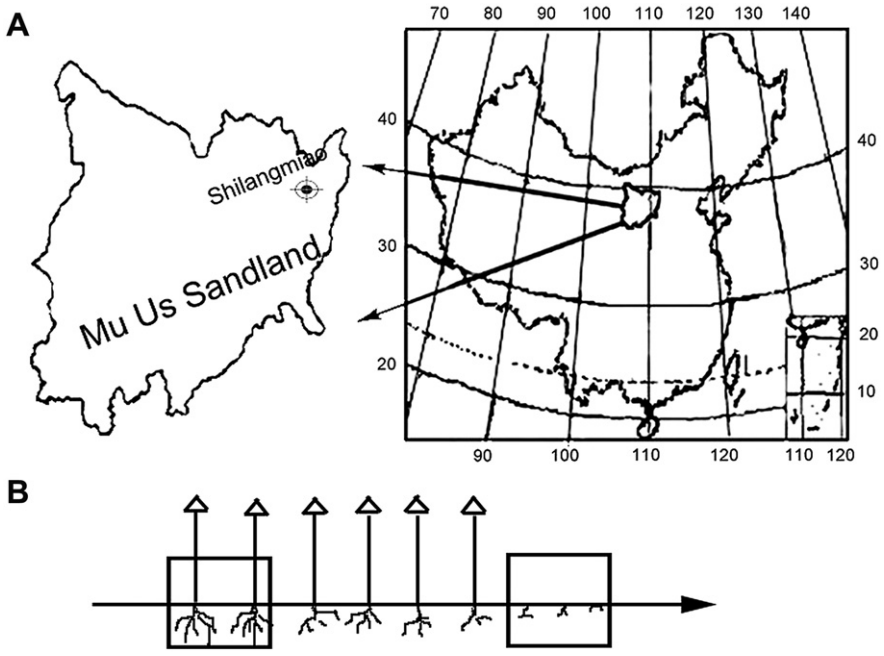

Fig. 1. (A) Sampling site at Shilangmiao in Mu Us sandland, China (B) The couple of plots. "The couple of plots" used for survey of AM fungi delimited by two real line boxes were designed in the axis of the rhizome of *P. villosa*. The right box represents the annual ramet type, including forthcoming ramets, and elongating, unbranched (no ramets and secondary rhizomes produced) young rhizome part. The left box, symbolizing the perennial ramet type over two years of age, is behind the right box basely successive at least four ramets. The straight line with arrow depicts the rhizome. The thin broken lines along the rhizome axis depict adventitious roots. The vertical line with the open triangle depicts the ramets. For the sampling period, the plots were physically connected to the bulk *P. villosa* standing behind them.