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Spatial Distribution of Glomalin-related Soil Proteins in Coniferous and Broadleaf mixed Temperate Forest

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Abstract

Glomalin-related soil protein (GRSP), as an important component of soil organic carbon (SOC) pool, is a glycoprotein produced by the hyphae of arbuscular mycorrhizal fungi (AMF), which play a vital role in carbon and nutrient cycling in forest ecosystem. Here we investigated the spatial distribution of GRSP in plant community of the dominated species not associated with AMF based on a typical coniferous and broad-leaved temperate forest in Mt. Changbai, Northeastern China. Spatial distribution of GRSP including easily extractable GRSP (EEG) and total GRSP (TG) is represented by Moran's *I* on different soil depth among seven soil layers of 0-5 cm, 5-10 cm, 10-20 cm, 20-30 cm, 30-50 cm, 50-70 cm and 70-100 cm. The concentrations of EEG and TG decreased with the increase of soil depth according to a logarithmic function. The Moran's *I* coefficient of GRSP was negative in all soil layers except TG in 20-30 cm and 50-70 cm soil layers. When EEG and TG were considered, the Moran's *I* coefficient was positive in majority of soil layers within the separation distance of less than 4 m but in soil layers of 10-20 cm and 20-30 cm for EEG and in 30-50 cm for TG. The largest Moran's *I* coefficient including EEG and TG was observed in the soil layer of 5-10 cm. The spatial distribution of GRSP was discrete in typical coniferous and broad-leaved temperate forest, and was affected by mycorrhizal colonization rate, soil organic carbon and total nitrogen.

Keywords: arbuscular mycorrhiza; GRSP; Mt. Changbai; spatial autocorrelation; temperate forest

Introduction

Glomalin-related soil protein (GRSP) is glycoprotein produced by the hyphae of arbuscular mycorrhizal fungi (AMF) that contains large content of metal ions (Gillespie *et al.*, 2011). GRSP is commonly found in various soils due to the ubiquitous colonization of AMF (Wright *et al.*, 1998a; Öpik *et al.*, 2013). The researchers have paid much attention to GRSP and AMF under global warming due to their importance in regulating soil carbon pool and slowing carbon turnover with 7-42 year (Steinberg *et al.*, 2003; Shi *et al.*, 2012). Meanwhile, GRSP is an important cementing agent in the formation of soil aggregates, which plays a major role in maintaining soil organic carbon (SOC) pool (Rillig *et al.*, 2001; Wright *et al.*, 2007), which contributed to SOC accumulation with more than 20 times higher than microbial biomass (Rillig *et al.*, 2001; Godbold *et al.*, 2006; Prescott, 2010; Guo, 2013). In addition, GRSP is one of the indicators of active nitrogen along with SOC (Rotter *et al.*, 2017). The GRSP in soil is classified into the total GRSP (TG) and the easily extracted GRSP (EEG) based on the different extracted methods (Wright *et al.*, 1998b; Rillig *et al.*, 2001).

Spatial heterogeneity is a soil property, which exists in all kinds of characteristics such as soil aggregate (Liu *et al.*, 2018), soil nutrient (Liu *et al.*, 2016), soil moisture (Yang *et al.*, 2017), etc. Therefore, it is inevitable that spatial heterogeneity also indwells on soil GRSP. He *et al.* (2010) showed that the dynamics of AMF and GRSP existed in high temporal and depth patterns, and was influenced by nutrient availability and enzymatic activity in Mu Us sandland. They also suggest that GRSP are useful indicators

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for evaluating soil quality and function. Wang *et al*. (2017) indicated that GRSP contributed more to carbon and nutrients in deeper soils, and differently associated with climates and soil properties in vertical profiles.

Spatial autocorrelation is one of the important forms for exploring the relationship between the research object and its location in spatial, which is an important indicator to test whether the investigated parameter is associated with itself in its adjacent spatial point (Anselin, 1983; Baltagi, 2005). Spatial autocorrelation has been used in lots of studies, such as soil organic carbon (Liang *et al*., 2007), soil moisture (Kim, 2013), plant diversity (Kim and Shin, 2016), microbial communities atop a debris-covered glacier (Darcy *et al*., 2017).

In temperate forests, the dominated plants usually form symbiosis with ecto-mycorrhizal fungi (Kohmei *et al*., 2018), and only a few species associate with AMF (Kubisch and Hertel, 2015; Veresoglou *et al*., 2017), although most understory plants could form a good symbiotic relationship with AMF (Mcguire, 2007). However, AMF could change the structural composition of the community to increase the community diversity when the subordinate plants have more affinity with AMF (Urcelay and Díaz, 2003; Shi *et al*., 2013a; Lin *et al*., 2015). Therefore, the distribution of GRSP and its mechanisms have attracted much attention. Wang *et al*. (2018) research showed that two different pathways for affecting the pool size of GRSP in mangrove ecosystems by directly via indigenous AMF propagules or via the GRSP transport and deposition by pore water and tides. Wu *et al*. (2014) indicated that root and soil GRSP exhibited spatial and temporal distribution patterns in citrus rhizosphere. In this study, we try to explore (i) the spatial distribution of soil GRSP and its spatial autocorrelation and (ii) the mechanisms of the distribution based on the analysis of mycorrhizal colonization rate, soil carbon and soil N concentration in temperate forest ecosystem.

Materials and Methods

Site description

This study was conducted in Mt. Changbai of northeastern China. Mt. Changbai is a major mountain in Northeastern Asia, located in the northeastern part of China, at the junction of Antu county, Fusong county and Changbai county in Jilin Province, with a total area of 196465 km^2 including forest of 16081 km^2, grassland of 5683 km^2 and the coverage rate reached 87.9% of forest(Wikipedia: https :// en. Wikipedia.org/__wiki/ Changbai_Mountain). Mt. Changbai is one of the intact natural ecosystems in China even all over the world. Furthermore, Mt. Changbai remains the large-scale native coniferous and broad-leaved mixed forest in the world, which maintains the richest biodiversity in the similar region of the northern hemisphere. The study site is a typical coniferous and broad-leaved cold temperate forest (42°24′N, 128°06′E) with the characteristics of altitude of 650~750 m, the mean annual temperature of 3.6 °C, the mean annual precipitation about 707 mm, and the soil type of dark brown forest soil of mountain. The dominant plants include *Pinus koraiensis*, *Betula platyphylla*, *Tilia amurensis*, *Picea jezoensis*, *Abies nephrolepis*, etc. are not associated with AMF (Hempel *et al*., 2003; Wang and Qiu, 2006; Shi *et al*., 2012).

Sample collection

Three 20 m × 20 m plots were designed in the typical coniferous and broad-leaved forest, and each of them was divided into 25 subplots with the size of 4 m × 4 m. Soil samples were collected from the centre of each subplot based on seven soil depth including 0-5 cm, 5-10 cm, 10-20 cm, 20-30 cm, 30-50 cm, 50-70 cm and 70-100 cm, respectively. The roots were isolated from each soil core. The soil samples were air-dried for measuring soil organ carbon, total nitrogen, EEG and TG.

Parameter analysis

The GRSP including EEG and TG was analyzed by the method proposed by Wright (1998b). SOC was measured by organic carbon analyzer (TOCV CPH, Shimadzu, Japan). Total nitrogen (TN) was determined by elemental analyzer dry burning (Elementar Analysensysteme, GmbH, Germany). AM colonization rate was determined according to the method described by Liu (2000) for evaluating the relationship between GRSP and AM colonization.

Spatial models

Spatial autocorrelation analysis was employed to test the attribute value of GRSP as to determine whether it was dramatically associated with its adjacent unit (Du *et al*., 2017). This analysis method can classify into positive correlation and negative correlation, positive correlation indicating that the attribute value of a spatial unit and its adjacent unit have the same change tendency, negative correlation indicating the opposite (Sadeq *et al*., 2016). The commonly spatial autocorrelation index is Moran's *I*.

Statistical analysis

The changes of GSPR including EEG and TG among seven different soil layers were analyzed by ANOVA. In addition, changes with depth of soil and their relationship with soil parameters were stimulated by regression. The spatial autocorrelation of GSPR was analyzed by Geoda software. Other statistical analyses were performed in SPSS 13.0 for windows.

Results

The distribution of GRSP with the change of soil depth

Both EEG and TG decreased markedly with the increase of soil depth, and the peak values in seven soil layers were 2.516 mg g^{-1} for EEG, 3.747 mg g^{-1} for TG (Fig. 1). The EEG and TG presented the similar trend whether the changes with soil depth. When their concentrations were considered among different soil depths, the differences of EEG and TG were significant among four soil layers in the surface 0-30 cm. However, no significant difference was observed among three soil layers in the depth of 30-100 cm. Furthermore, the EEG and TG declined with the increase of soil depth according to a logarithmic function, which show that the changes of soil depth account for 94.67% and 93.49% for the variation of EEG and TG, respectively.

Spatial autocorrelation of GRSP at different soil layers

The Moran's *I* coefficient is employed for exploring the spatial autocorrelation of GRSP in seven soil layers (Table 1). The Moran's I coefficient of EEG was negative in all soil depth', which indicate that the distributed characteristics of EEG are discrete in all seven soil layers. That is to say, EEG concentrations between two adjacent areas are not correlative. As to TG, the similar trend of Moran's I coefficient was observed in all soil depth expect for in soil layers of 20-30 cm and 50-70 cm.

Further, the change characteristics with distance of EEG and TG at different soil layer were presented by global Moran's *I* coefficient (Fig. 2). There are positive Moran's *I* coefficient of EEG expect for 10-20 cm and 20-30 cm soil layers when the separation distance is less than 4 m (Fig. 2a). There are negative or almost zero Moran's I coefficient of EEG expect 5-10 cm soil layer when the separation distance is less than 8 m. When less than 12 m of the distance is considered, there are negative or almost zero Moran's *I* coefficient of EEG expect 20-30 cm and 30-50 cm soil layers. Further, when the distance increases to less than 16m, negative Moran's *I* coefficient is observed on EEG in the four soil layers of 5-50 cm. There are negative Moran's I coefficient of EEG expect for in 10-20 cm soil layer when the separation distance is less than 20 m. The changes of Moran's *I* of TG are smaller in all soil layers during the separation distance of 0 to 16 m compare to between 16 and 20 m. The largest Moran's I coefficient of TG was observed in soil layer of 5-10 cm when seven soil layers are considered (Fig. 2b).

Table 1. Level autocorrelation of EEG and TG

Soil layer/cm	EEG（Moran's *I*）	TG（Moran's *I*）
0-5	-0.1270	-0.1645
5-10	-0.1129	-0.0378
10-20	-0.1276	-0.1445
20-30	-0.0455	0.0049
30-50	-0.0889	-0.0356
50-70	-0.0829	0.0858
70-100	-0.0650	-0.1060

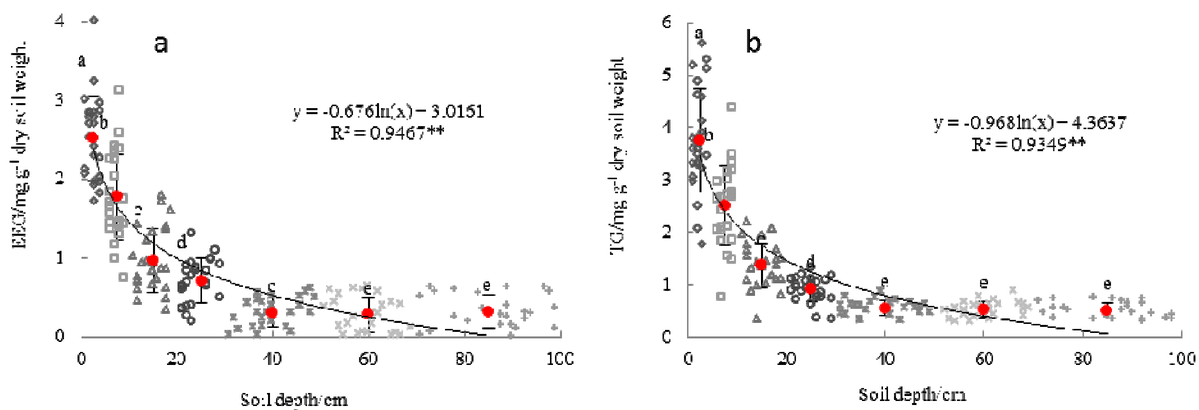

Fig. 1. The concentration of EEG (a) and TG (b) in seven different soil layer and their changes with depth of soil

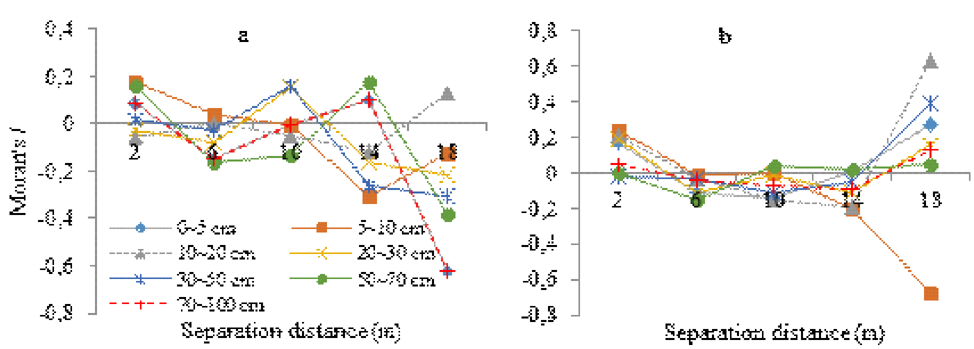

Fig. 2. Variation of Moran's *I* with distance of EEG (a) and TG(b) at different soil layers

Relationship between GRSP and soil parameters

The relationship between GRSP (EEG or TG) and mycorrhizal colonization can be stimulated by a linearly equation (Fig. 3). The mycorrhizal colonization accounts for the variation of EEG and TG of 19.55% and 25.85%, respectively. The concentrations of EEG and TG increased 0.1248 and 0.2181 mg/g, respectively when mycorrhizal colonization increases 1%.

Comparing to mycorrhizal colonization, SOC accounts for more variation of EEG and TG with the explanation rate of 71.76% and 70.59%, respectively (Fig. 4). EEG and TG presents similar trend with the increase of SOC. The similar trends of EEG and TG are observed with SOC when soil TN is considered (Fig. 5). Further, the explanation degree of soil TN to EEG and TG is almost same, although the increasing rate of EEG and TG is different with the increase of TN.

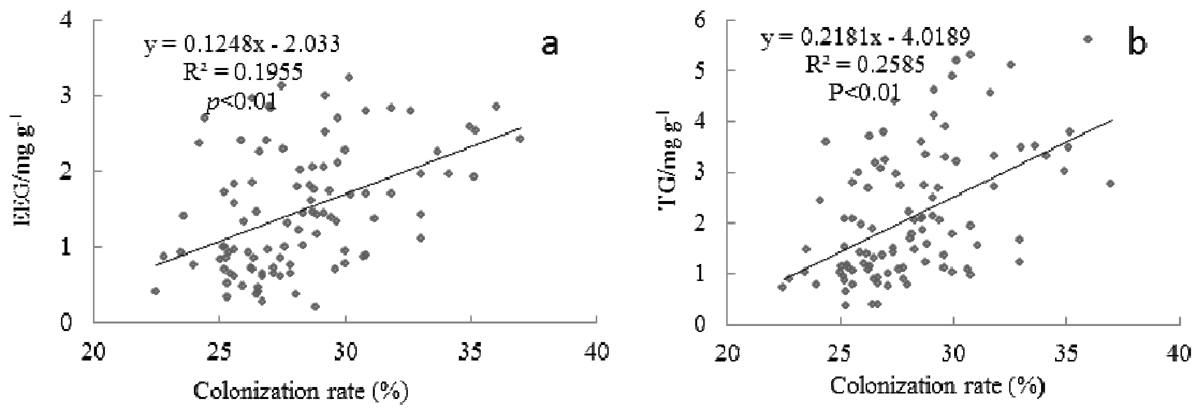

Fig. 3. The relationship between EEG (a) and TG (b) with colonization rate

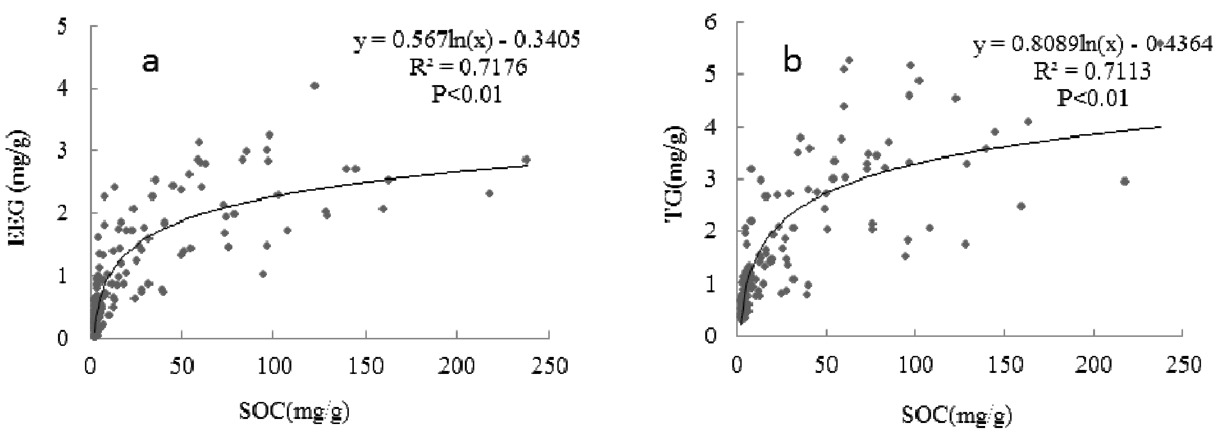

Fig. 4. Relationship between EEG (a) and TG (b) and soil organic carbon (SOC)

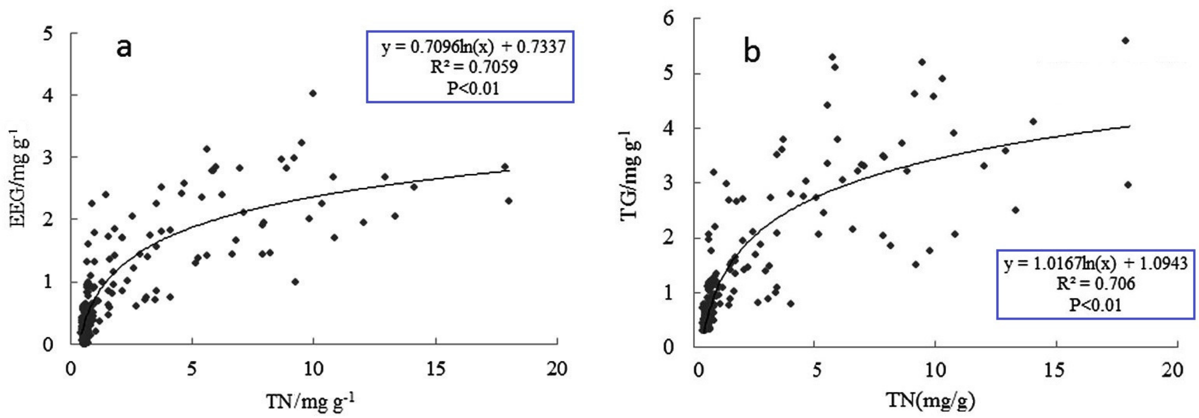

Fig. 5. Relationships between EEG (a) and TG (b) and (TN)

Discussion

Spatial autocorrelation is a spatial analysis method commonly used in all kinds of fields, such as environment (Shen *et al*., 2019), plant diversity (Kim and Shin, 2016), medicine (Akmatov *et al*., 2018), traffic (Blazquez *et al*., 2018). Certainly, spatial autocorrelation was usually used to explain the spatial variation of soil characteristics, such as soil moisture (Kim, 2013), soil organic carbon (Liang *et al*., 2007), soil microbial community (Deakin *et al*., 2018). Spatial autocorrelation also used in mycorrhizal fungal diversity, such as AMF communities (Horn *et al*., 2014) and spatial variation (Shi *et al*., 2013b), but there is little study in GRSP. GRSP as an exclusive organic substance secreted by AMF, which is a main indicator for studying soil carbon pools and nitrogen pools (Jing *et al*., 2017). Therefore, we studied the spatial distribution of GRSP in soil on temperate forests where hold huge carbon.

Researches have shown that the soil depth has a significant effect on GRSP (Roldan and Salinas, 2007; Wang *et al*., 2017). In this study, we study the concentration of GRSP (EEG and TG) at a 0-100 cm soil profile, which showed that GRSP exhibited a similar relationship in a shallow 30 cm soil profile. This finding is consistent with previous studies that exhibited obvious vertical decreasing pattern in a shallow 40 cm soil profile (Wu *et al*., 2012; Wang *et al*., 2017). However, our results showed that no difference was observed both EEG and TG among three soil layers from 40 to 100 cm, which conflicted with the study made by Wang *et al*. (2017) in farmland. This possible reason is due to the ecological type. The root is shallow in farmland compared to forest, which lead to the rapid decrease of GRSP in deeper soil. Further, this also caused the changes of GRSP according to log function in the current study rather than linear function (Wang *et al*., 2017). Anyway, the decreasing trend of GRSP with the soil deepening is consistent with previous majority studies (Wang *et al*., 2017; Wu *et al*., 2012).

The autocorrelation of soil nutrient parameters, even glomeromycotan family composition has been analyzed (Stürmer *et al*., 2018). However, this study is the first to explore the autocorrelation of GRSP in different soil depths in our knowledge, although GRSP is an important indicator in the process of driving spatial patterns of natural ecosystems by AMF in different soil depth and climate (Chaudhary *et al*., 2014). The positive Moran's I presented significant spatial autocorrelation, as the distance increased both for the family composition. Conversely, the negative showed sites tend to present less similarity in GRSP concentration. our result show that there are positive autocorrelation of EEG when the separation distance is less than 4 m expect 10-20 cm and 20-30 cm soil layers (Fig. 3), which indicated the aggregated distribution of GRSP. The similar finding is obtained in TG when the separation distance is less than 4 m expect 30-50 cm soil layer.

As the important indicator of the form of symbiotic mycorrhizas between AMF and host plants, mycorrhizal colonization status is usually measured in numerous studies (Wang *et al*., 2011; Cui *et al*., 2013; Shi *et al*., 2017). Our result also suggested that soil GRSP increases with the enhancement of mycorrhizal colonization according to the linear function based on the regression analysis, which is support the previous conclusion drawn by Liu et al. (2013) based on different soil layers of *Robinia pseudoacacia* plantation on the hilly region of the Loess Plateau. This should tend to be understood because GRSP is a kind of exclusive organic substance secreted by AMF. Many studies have indicated that SOC and TN can affect the concentration of soil GRSP (Wu *et al*., 2014; Xiao *et al*., 2019), which is also testified further in this study. Moreover, as a component of SOC, GRSP also affect SOC and TN each other. Zhang *et al*. (2017) suggested that GRSP probably regulate the resistance of SOC sequestration in tropical forests, especially in the planted and secondary forests. However, Hodge *et al*. (2001) demonstrated AMF accelerates decomposition and acquires nitrogen directly from organic material, which confirmed that AMF has implications for N cycling based N acquisition from organic material. Certainly, the positive correlations between soil N and GRSP have been testified in previous studies (Fokom *et al*., 2013; Wang *et al*., 2017).

Conclusions

The concentration of GRSP including EEG and TG decreased with the increase of soil depth according to a logarithmic function. There are not spatial autocorrelation of GRSP in all soil layers. When EEG and TG were considered, the Moran's I coefficient was positive in majority soil layers with the separation distance is less than 4 m. The spatial distribution of GRSP is discrete in typical coniferous and broad-leaved temperate forest and is affected by mycorrhizal colonization rate, SOC and TN.

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Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

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