Long-term overgrazing reduces glomalin-related soil protein in alpine grassland

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Abstract

Glomalin-related soil protein (GRSP) is a recalcitrant glycoprotein mainly produced by arbuscular mycorrhizal fungi and contributes to soil carbon sequestration. Human activities (grazing, fertilization, etc.) can change plant productivity, soil carbon pool, and microbial community in an alpine grassland ecosystem. However, no study has reported on the effect of human activities on GRSP. Besides, the effect of the interaction between environmental factors and human activities on GRSP is unknown. This study assessed GRSP response to grazing intensity gradients and fertilization in an alpine grassland (Qinghai-Tibet Plateau). The result showed that livestock grazing changed GRSP stability in alpine grassland. Moreover, the content of total GRSP and easily extractable GRSP were gradually decreased with increasing grazing intensities in both surface and subsurface soils. GRSP was highly positively correlated with soil organic carbon (SOC), total nitrogen (TN), and available phosphorus (AP) but negatively correlated with the soil inorganic carbon (SIC) and PH ($P<0.001$). GRSP promoted SOC by 3.7-14.18%. In contrast, N and P addition for five years did not affect SOC and GRSP contents. Therefore, GRSP is a stable organic carbon essential for SOC fixation. Short-term distractions cannot affect GRSP stability, while long-term overgrazing can gradually reduce GRSP stability. This study provides new insights into soil carbon pool and ecological stoichiometry in the grassland succession process on the Qinghai-Tibet plateau.

1. INTRODUCTION

Glomalin is a glycoprotein mainly produced by arbuscular mycorrhizal fungi (AMF) (Wright & Upadhyaya, 1996). It is a hyphal wall component that accumulates in soils and has a slow turnover (Rillig et al., 2001). Glomalin is composed of carbon (C) (36-59%), nitrogen (N) (3-5%), and iron (Fe) (1-9%). Glomalin mainly increases SOC pools and improves soil aggregates (Rillig et al., 2001; Wang et al., 2018). C content in glomalin represents 4~5% of the total soil C, which is much higher than soil microbial biomass C (Zhu and Miller, 2003). Glomalin in soils is called glomalin-related soil protein (GRSP) (Rillig, 2004), which is classified into two fractions: easily extractable GRSP (EE-GRSP) and total GRSP (T-GRSP).

GRSP, as an insoluble and hydrophobic proteinaceous substance, can act as long as 6~42a in soil (Rillig et al., 2001). However, it is unclear whether GRSP changes with the environment and human activities. Grazing via domestic ungulates is the main human activity on grassland ecosystems that affects ecosystem processes and functions (Ebrahimi et al., 2016). The alpine grassland ecosystem dominates the Qinghai-Tibet Plateau, covering over 1.28×10^6km^2 (50.9% of the entire plateau area) and contains enormous organic carbon. In recent decades, alpine grasslands have experienced rapid degradation (Xiong et al., 2014) mainly due to livestock overgrazing and dry climate (Dong et al., 2013). Excessive livestock grazing affects nutrient pool and cycling, net primary productivity, vegetation composition, belowground biomass productivity, and associated changes in the soil microbial community (Cao et al., 2004; Veen et al., 2014). Chemical fertilizers (nitrogen (N) and phosphorus (P)) are widely used to enhance grassland productivity yields and soil fertility for grazing (Hacker et al., 2011; Zhou et al., 2015). N and P are the main limiting elements controlling the productivity and functioning of the Tibetan alpine grassland ecosystems (Yang et al., 2020; Dong et al., 2020).

Grazing and fertilization can change plant productivity, soil carbon pool, and microbial community of grassland ecosystem, thus affecting the protein content secreted by soil microorganisms. Wang et al. (2014) showed that overgrazing significantly decreases the total mycorrhizal colonization and GRSP content in grassland. Moreover, nitrogen fertilizer (10 g/m^2) can significantly increase T-GRSP content but has no significant effect on the EE-GRSP in the Prairies (Wilson et al., 2009). Long-term phosphate fertilizer application significantly increases GRSP content compared with the control (Johnson et al., 2003; Chagnon and Bradley, 2013). Various components of GRSP have different responses to fertilization (Turgay et al., 2014; Zhang et al., 2015), possibly because different soil nutrient contents have different plant productivity and decomposition rates of GRSP (Rillig et al., 2002; Steinberg and Rillig, 2003).

The alpine grassland ecosystem of the Qinghai-Tibet Plateau is extremely fragile, slow to recover, and greatly affected by external disturbance. No study has reported on the effect of human activities on GRSP. Besides, the influence of the interaction between environmental factors and human activities on GRSP is unknown. The spatial and temporal variation and the mechanistic of stable structural GRSP caused by grazing and fertilization are also unclear. Herein, GRSP response to grazing intensity and fertilization was studied.

Herein, an alpine meadow with grazing intensity gradients and different fertilization practices were used. The study aimed to: (1) determine GRSP content variation in response to different gradients of grazing intensity and different fertilization (N and P) treatments; (2) Identify interrelationships among GRSP, SOC, and edaphic properties in response to human activities. This study provides insights into the stable organic carbon function of GRSP for improving rangeland management practices on the Qinghai-Tibet plateau.

2. MATERIALS AND METHODS

2.1 Study site and sampling

The study was conducted at Haibei Alpine Meadow Ecosystem Research Station, belonging to the Northwest Institute of Plateau Biology of the Chinese Academy of Sciences. The site is situated within a large valley surrounded by the Qilian Mountains, in the northeast of the Tibetan Plateau (37°29'-37°45' N, 101°12'-101°23' E, altitude of 3200-3500 m a.s.l.). The area has a typical plateau continental climate, with mean annual temperatures and precipitation of −1.7 °C and 582 mm, respectively. More than 80% of precipitation falls from May to September during the plant growing season. The site has Mat Cry-gelic Cambisols and silty clay loam soils, based on Inceptisols of the U.S. soil taxonomic system.

The dominant population of alpine grassland gradually changes from Gramineae-*Kobresia humilis*community to *Kobresia humilis* and *Kobresia pygmaea*communities under long-term overgrazing. Furthermore, long-term overgrazing increases root accumulation and decreases surface soil bulk density and the available soil nutrients, resulting in soil nutrient imbalance between demand and supply. Moreover, the thickening of the mattic epipedon reduces the water infiltration capacity. It forms dark spots and local death in *Kobresia pygmaea* community, which eventually evolve into "Black-soil-type" degraded land. Therefore, the experimental sites with different grazing intensities were defined as Gramineae-*Kobresia humilis* community (GK), *Kobresia humilis* community (K), thickening-in-mattic epipedon of *Kobresia pygmaea* community (KT), cracks-in-mattic epipedon of *Kobresia pygmaea* community (KC), and Forb-black soil type grassland (FB) (Table 1). The sites were named after the succession of the plant community after a long period of grazing lambs and sheep. The grazing intensities of the GK, K, KT, KC sites were 3.65, 7.50, 11.25, and 13.00 sheep ha^{-1}, respectively. FB caused substantial degradation and could not be used for additional grazing. The sites were used as winter pastures with a grazing period from 20th September to 20th May. Each site was approximately 30-40 ha, and was fenced. The alpine meadow has experienced different grazing intensities since 1995. The aboveground plant tissues were not removed or consumed by grazing in the summer since the meadow was used as winter pasture.

The nutrient addition experiment was conducted in the Gramineae-*Kobresia humilis* community in Haibei station to determine the effect of nutrients on the grassland. The above experiment was a completely randomized block group experimental design, with four treatments: nitrogen addition (N), phosphorus addition (P), nitrogen and phosphorus mixed addition (NP), and control (CK). The treatments were randomly arranged in six plots (six repeats) (24 plots), measuring 6 m×6 m, with protection lines between all the plots. N fertilizer (100 kg/ (hm^2·year)) and P fertilizer (50 kg/(hm^2·year) were added in the form of urea, and heavy superphosphate, respectively. NP was added as a mixture of N and P fertilizers, and the control treatment had no nutrients. Fertilizing was conducted in the middle of the planting season, rainy or sunny evenings. Fertilizer was evenly distributed in the corresponding area by hand. Fertilizer was applied twice in each district to ensure the uniformity of fertilization. Nutrients were added from 2011 to 2015, on 1st June, 1st July, and 1st August of each year.

Five meadow sites under different grazing intensities and nutrient addition experimental sites were selected on 23rd July 2015 to determine GRSP and soil properties. Each site was divided into six plots. Soil was collected from five randomized points in each plot at depths of 0-10 cm and 10-20 cm. The soil samples of each soil depth in every plot were mixed into one sample, yielding 32 (five succession stages) and 48 (nutrient addition) samples, then kept on ice before being stored at 4 ^{o}C in the laboratory as quickly as possible. Fresh soil was passed through a 2-mm mesh sieve after removing visible litter, roots, and stones. The soil sample was thoroughly mixed and stored at 4 °C for the determination of the biogeochemical properties.

2.2 GRSP determination

The glomalin content was estimated by measuring the EE-GRSP and T-GRSP (Rillig et al., 2002). The EE-GRSP was extracted from the soil samples using 20 mM sodium citrate at pH 7.0 and 121 °C for 30 min, while the T-GRSP was extracted using 50 mM sodium citrate at pH 8.0 and 121 °C for 60 min (repeated 4-5 times). The extracts were centrifuged at 10 000 × g to remove soil particles. The protein content of the supernatant was quantified using the Bradford dye-binding assay with bovine serum albumin as the standard (Rillig et al., 2001; Rillig et al., 2002).

2.3 Soil properties analyses

A PHS-3C pH meter (Shanghai Lida Instrument Factory, Shanghai, China) was used to measure the soil pH via the potentiometric method in a soil-to-water ratio of 1:2.5 (w/v). The soil moisture was gravimetrically measured. An elemental analyzer (2400 II CHNS/O, Perkin-Elmer, Danbury, CT, USA) was used to measure the soil total carbon (C) and total nitrogen (N) via dry combustion at a combustion temperature of 950 °C. The soil inorganic C was volumetrically measured using an inorganic C analyzer (Calcimeter 08.53, Eijkelkamp, Giesbeek, Netherlands). The soil organic C content was indirectly determined as the difference between the total C and inorganic C contents. An automated segmented flow analysis (SmartChem140, AMS Systea, Roma, Italy) was used to colorimetrically measure the ammonium (NH_4^+) and nitrate (NO_3^-) contents in the soil via the salicylate/dichloroiso-cyanuric acid and cadmium column/sulphanilamide reduction methods, respectively. The available phosphorus (P) was extracted using 0.5 mol L^{-1} $NaHCO_3$ (pH 8.5) and measured by a 722s visible light spectrophotometer (Shanghai Precision and Scientific Instrument Corporation, Shanghai, China).

2.4 Statistical analysis

The relationship between GRSP and biochemical features was assessed in SPSS 20 (IBM, Inc., Armonk, NY, USA). One-way analysis of variance (ANOVA) and Tukey's HSD tests was used to compare the variations in soil GRSP and biochemical properties among the five grazing successive stages and nutrient addition treatments. The figures were mapped using Sigma Plot 12.5. Redundancy Analysis (RDA) was conducted using Canoco 5.0.

3. RESULTS

3.1 Effects of grazing on the GRSP concentration

The concentration of T-GRSP and EE-GRSP were determined for each grazing succession stage (Fig. 1). Analysis of variance showed that the mean concentrations of T-GRSP and EE-GRSP in soils were significantly different ($P < 0.05$) between the surface (0-10 cm) and subsurface (10-20 cm) soils. T-GRSP (10.42% (FB)-81.25% (KC)) and EE-GRSP (12.63% (FB)-70.31% (K)) were higher in the surface than in the subsurface soil. T-GRSP and EE-GRSP were highest in GK community sites (14.71±0.67 and 3.69±0.15 mg/g, respectively, in surface soils, and 8.85±0.92 and 2.30 ±0.19 mg/g, respectively, in subsurface soils). The concentration of T-GRSP and EE-GRSP in surface soils had a significant decreasing trend from GK, K, KT, KC to FB. However, the contents were not significantly different among K, KT, and KC. T-GRSP in surface soils was lowest in KT and FB, while EE-GRSP was lowest in KC community sites. Overall, GRSP contents gradually decreased with increasing intensities of grazing.

3.2 Effects of grazing on the soil physicochemical properties

The soil physicochemical properties among five successional stages in alpine grassland are shown in Table 1. Except for soil pH and inorganic carbon, all soil index contents were higher in the surface layer than in the subsurface layer. The soil water content, ammonium nitrogen, total nitrogen, total carbon, and organic carbon were successively decreased from GK to FB. In contrast, the inorganic carbon content increased, and the soil quality gradually calcified. Soil nitrate, nitrogen, and available phosphorus were highest in GK and K but showed a decreasing trend in KT and an increasing trend in the degraded grassland.

3.3 Relationship between GRSP and soil nutrients

Correlation analysis showed that the T-GRSP and EE-GRSP were highly positively correlated with the contents of SOC, TC, TN, and AP but negatively correlated with SIC and PH ($P<0.001$) (Fig. 2). The redundancy analysis (Fig. 3) results indicated that SOC is the main impact factor for T-GRSP and EE-GRSP. The explains of SOC to the GRSP was 84.8%, and the contribution of SOC to the GRSP was 92.9%. TC was removed since TC and SOC are collinear. Redundancy analysis and linear regression analysis (Fig. 4) showed that SOC, TN, and AP were positively correlated with both T-GRSP and EE-GRSP ($P<0.01$).

3.4 Effects of N and P addition on GRSP and SOC concentrations

T-GRSP and EE-GRSP contents were determined after N, P, NP, and control treatments (Fig. 5). Similarly, the mean concentrations of T-GRSP and EE-GRSP were significantly different ($P < 0.05$) between the surface and subsurface soils. However, T-GRSP and EE-GRSP contents were not significantly different between the surface and subsurface soils after N, P, NP, and the control treatments for five years. Short-term nutrient addition did not affect the soil GRSP. Meanwhile, the SOC content was also not significantly affected. Furthermore, SOC was positively correlated with both T-GRSP and EE-GRSP ($P<0.001$) (Fig. 6). These results show that short-term N and P addition cannot improve soil organic carbon pool.

4. Discussion

4.1 Grazing and GRSP

Livestock grazing is the major human activity in grassland ecosystems that alters grassland production, biodiversity, soil C storage and ultimately causes grassland degradation (Wang et al., 2020; Liu et al., 2021). This study used Kobresia meadow, the main type of alpine meadow widely distributed across the middle and eastern part of the Qinghai-Tibetan Plateau (He et al., 2016), and causes various degenerate successions. Gramineae-*Kobresia humilis*community is a typical alpine meadow that is made up of a double layer structure (upper layer, gramineous forage grass, and lower layer,*Kobresia humilis* community). However, long-term overgrazing affects gramineous forage growth and reproduction due to livestock feeding and trampling. The sedge plants of the root system rapidly grow, increasing the root-soil ratio and root cap ratio. Meanwhile, the mattic epipedon cracks due to the dry root environment, corrosion caused by harsh weather, and disturbance by the small mammalian herbivores, thus gradually degrading the alpine meadow (Cao et al., 2018). Therefore, the alpine meadow successively undergoes the degradation and succession process (GK, K, KT, KC, and FB). Herein, the aboveground plant biomass and diversity were decreased with the intensification of grazing. These results show that soil properties change with the grazing gradients. For instance, the soil water and nutrient content decreased from the GK community to the FB community and thus affecting the C and GRSP contents. GRSP contents were gradually decreased with increased grazing intensities. Chen et al. (2018) also indicated that GRSP in soil (2–15 mg/g) contains 5–10% of total SOC. Herein, GRSP concentrations were also within the above range with 3.7-14.18% SOC. GRSP is a recalcitrant compound and a stable form of SOC (Chen et al., 2018; Wang et al., 2018). However, disturbance from outside environments affects GRSP (Zhang et al., 2015; Lozano et al., 2016). Grazing affects GRSP stability and the fixation of soil organic matter in alpine grassland. Moreover, the succession process of alpine grassland causes various soil organic carbon succession.

4.2 N and P fertilization and GRSP

Human activities, such as grazing and nutrient addition, cause environmental changes (Zhang et al., 2015). Herein, grazing changed environmental conditions and soil GRSP content. Also, for five years, N and P addition increased the available N and P content and activities of some enzymes (Sun et al., 2016). However, the addition did not affect SOC and GRSP contents. Previous reports indicated that N fertilization(10g/m^2)for 0.3-1.6 years does not significantly affect GRSP (Garcia et al., 2008). Zhong et al. (2017) also found that N addition (0–300 kg N ha-1 y-1) for one year does not significantly influence SOC in any size aggregate fractions. Herein, N and P fertilization for four years showed a similar effect. This may be due to the relatively long turnover of GRSP and the shorter duration of fertilization, which usually has a delayed effect. Therefore, a longer duration of nutrients fertilization is required to detect changes in GRSP concentrations. In contrast, Zhang et al. (2015) indicated that continuous N addition for five years increases T-GRSP in the top 5 cm soil by 30%, but it does not affect EE-GRSP content. Other studies have shown that GRSP contents increases after N addition (Sun et al., 2018) and manure input (Xie et al., 2015). These inconsistencies may be due to the initial soil nutrient element content (Treseder et al., 2007) and nutrient element addition level and duration (Garcia et al., 2008; Sun et al., 2018).

4.3 Relationships among GRSP, SOC, and soil variables

GRSP content was significantly positively correlated with SOC and TN. GRSP comprises carbon and nitrogen and is highly correlated with the carbon and nitrogen content in the soil (Vasconcellos et al., 2016). Other studies have also shown a positive relationship between GRSP with SOC and TN (Zhang et al., 2015; Wang et al., 2018; Qiao et al., 2019). C and N contents in glomalin represent 4–5% of the total soil C and N (Rillig et al., 2001). GRSP content was associated with SOC probably because GRSP is also a component of soil organic matter (Rillig et al., 2001). Besides, GRSP and SOC depend on similar ecosystem component (photosynthetic productivity) and contribute to similar ecosystem functions (feeding decomposer community and formation of soil aggregates) (Kumar et al., 2018). Moreover, GRSP promotes soil C pools in native grasslands for a soil binding agent (Purin et al. 2006). In summary, GRSP regulates the accumulation and circulation of SOC in alpine grassland soils. Meanwhile, GRSP is an N-linked glycoprotein that increases soil N levels (Wright and Upadhyaya, 1998).

GRSP content was negatively correlated with pH, consistent with other studies (Wang et al., 2014; Wang et al., 2017). Antibus et al. (2006) also indicated that EE-GRSP is negatively correlated with pH. Previous studies have also shown that soil pH regulates GRSP accumulation, precipitated under acidic conditions, and soluble under alkaline pH conditions (Singh et al., 2017). Decreased soil pH facilitates GRSP accumulation (Wang et al., 2017), explaining the negative correlation between GRSP and pH.

5. CONCLUSION

This study demonstrated that livestock grazing can change GRSP stability in alpine grassland. Also, T-GRSP and EE-GRSP contents were gradually decreased as grazing intensity increased. Grazing also affects SOC fixation. The succession process of alpine grassland also causes various soil organic carbon succession. GRSP is strong positive correlated with SOC and TN, and GRSP contributing 3.7-14.18% of SOC. N and P nutrient addition for five years increases the available N and P content, but it does not affect SOC and

GRSP contents. Therefore, GRSP is a stable organic carbon and regulates SOC fixation. Moreover, short-term grazing cannot affect GRSP stability, but long-term overgrazing can gradually reduce GRSP stability. This study showed the impact of grazing and fertilization on GRSP and another indicator of stable soil carbon. Therefore, it provides insights into the changes of soil carbon pool and ecological stoichiometry during grassland succession on the Qinghai-Tibet plateau.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to claim in the publication of this research.

AUTHOR CONTRIBUTIONS

Qian Li: Investigation (Lead); Methodology (Lead); Writing-original draft (Lead); Writing-review & editing (Lead).**Guangyue Zhao:** Formal analysis (Lead); Investigation (Equal); Writing-original draft (Equal)**.** **Dawen Qian:** Project administration (Lead); Software (Lead); Writing-original draft (Equal).**Yangong Du** : Methodology (Equal); Resources (Lead); Writing-review & editing (Equal). **Xiaowei Guo** : Investigation (Equal); Software (Equal). **Bo Fan** : Investigation (Supporting); Writing-original draft (Equal). **Guangmin Cao** : Supervision (Equal); Visualization (Equal).

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Fig. 1. T-GRSP and EE-GRSP contents in different successional stages of alpine grassland. Different capital letters indicate significant differences between two soil layers in the same successional stages, and different lowercase letters indicate significant differences among different successional stages in the same soil layers at $P<0.05$ level. GK: Gramineae-*Kobresia humilis* community; K: *Kobresia humilis* community; KT: Thickened mattic epipedon of *Kobresia pygmaea* community; KC: Cracked mattic epipedon of *Kobresia pygmaea* community; FB: Forb-black soil type grassland.

Table 1 Soil physicochemical property in different succession states of alpine grassland

Soil physicochemical property	Soil layers	GK	K	KT	KC	FB
pH	0-10 cm	7.10 (0.02)^{a}	7.55 (0.36)^{a}	7.30 (0.09)^{a}	7.55 (0.30)^{a}	7.61 (0
	10-20 cm	7.69 (0.43)^{a}	8.08 (0.11)^{a}	7.99 (0.09)^{a}	8.18 (0.13)^{a}	7.99 (0
Soil moisture (%)	0-10 cm	53.95 (3.27)^{a}	46.15 (4.58)^{b}	45.04 (3.43)^{b}	37.05 (0.63)^{c}	20.74 (
	10-20 cm	38.80 (3.01)^{a}	37.37 (1.96)^{ab}	36.74 (2.61)^{ab}	34.17 (0.49)^{b}	26.09 (
Ammonium nitrogen (mg*kg^{-1})	0-10 cm	14.27 (1.32)^{a}	12.46 (0.92)^{b}	2.95 (1.36)^{b}	12.25 (2.00)^{b}	11.42 (
	10-20 cm	11.08 (0.81)^{a}	10.07 (1.72)^{a}	8.91 (0.47)^{a}	8.97 (0.18)^{a}	11.05 (
Nitrate nitrogen (mg*kg^{-1})	0-10 cm	24.67 (4.26)^{a}	30.81 (1.34)^{a}	13.63 (2.82)^{b}	23.52 (1.88)^{a}	28.85 (
	10-20 cm	22.46 (4.96)^{b}	22.08 (3.27)^{b}	12.70 (0.80)^{c}	13.57 (2.41)^{c}	27.80 (
Available phosphorus (mg*kg^{-1})	0-10 cm	4.50 (0.28)^{a}	3.53 (0.49)^{b}	2.56 (0.07)^{d}	3.22 (0.07)^{bc}	2.87 (0
	10-20 cm	2.29 (0.57)^{a}	1.12 (0.46)^{bc}	0.94 (0.08)^{c}	1.15 (0.07)^{b}	1.94 (0
Total nitrogen (g*kg^{-1})	0-10 cm	12.47 (0.70)^{a}	9.47(0.90)^{b}	9.07 (1.01)^{b}	9.63 (0.32)^{b}	3.88 (0
	10-20 cm	7.63 (1.01)^{a}	7.10 (0.95)^{a}	6.87 (0.90)^{a}	6.60 (0.95)^{a}	3.38 (0
Total carbon (g*kg^{-1})	0-10 cm	130.00 (0.87)^{a}	92.93 (10.66)^{b}	92.23 (6.82)^{b}	84.83 (14.63)^{b}	61.38 (
	10-20 cm	68.73 (9.69)^{a}	67.07 (8.58)^{a}	59.23 (12.76)^{ab}	60.50 (8.86)^{ab}	54.65 (
Inorganic carbon (g*kg^{-1})	0-10 cm	0.25 (0.10)^{c}	2.76 (0.71)^{b}	0.36 (0.29)^{c}	1.77 (1.19)^{b}	11.10 (
	10-20 cm	2.83 (1.57)^{d}	7.63 (0.89)^{c}	6.79 (3.42)^{c}	9.75 (4.48)^{b}	11.55 (
Organic carbon (g*kg^{-1})	0-10 cm	129.75 (11.94)^{a}	90.17 (11.24)^{b}	91.87 (6.64)^{b}	83.07 (13.51)^{b}	50.28 (
	10-20 cm	65.90 (10.35)^{a}	59.44 (9.06)^{a}	52.44 (14.64)^{ab}	50.75 (13.27)^{ab}	43.10 (

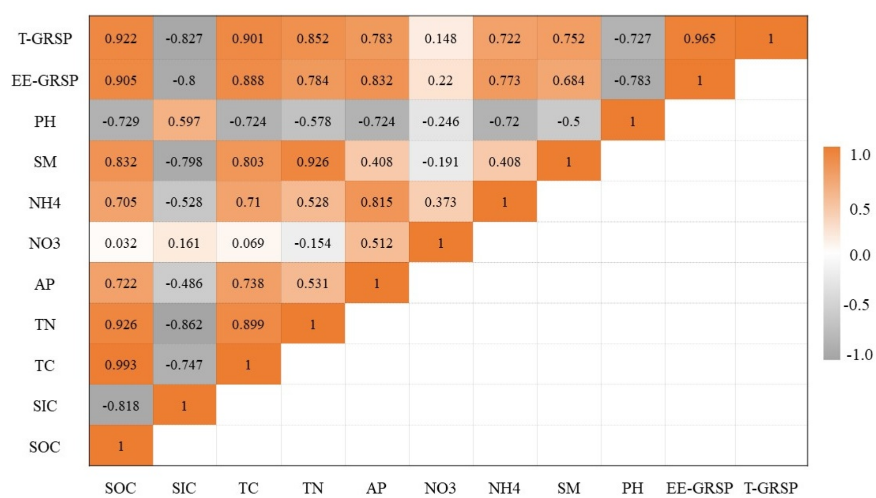

Fig. 2. Correlation analysis between GRSP contents and soil physicochemical properties

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Fig. 3. Redundancy analysis for GRSP and soil physicochemical properties

Fig. 4. Linear regression analysis of GRSP (T-GRSP and EE-GRSP) with Soil organic carbon (SOC), Total nitrogen (TN), and available phosphorus (AP).

Fig. 5. T-GRSP and EE-GRSP contents in control and fertilization (N, P, and NP) treatments. Different capital letters indicate significant differences between two soil layers in the same successional stages. Different lowercase letters indicate significant differences among various successional stages in the same soil layers at 0.05 level.

Fig. 6. (a) The soil organic carbon content in control and fertilization (N, P, and NP) treatments. Different capital letters indicate significant differences between two soil layers in the same successional stages. Different lowercase letters represent significant differences among different successional stages in the same soil layers at 0.05 level. (b) Relationship of GRSP (T-GRSP and EE-GRSP) with Soil organic carbon (SOC).