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Controlling soil total nitrogen factors across shrublands in the Three Rivers Source Region of the Tibetan Plateau

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Alpine shrublands in the Three Rivers Source Region (TRSR) store substantial soil total nitrogen (N); however, limited information is available regarding its storage and controlling factors. To quantify the storage and controlling factors of soil total N stock, we analysed 66 soil profiles from samples obtained from 22 shrubland sites located across the TRSR on the Tibetan Plateau. Analytical methods, such as ordinary least squares regression, one-way analysis of variance, curve estimation, and variation partitioning were used to evaluate the effects of soil characteristics (soil organic carbon), vegetation characteristics (community types and ground cover of shrublands), climatic factors (mean annual temperature – MAT), and topographical features (slope) on soil N stock. Our results showed that soil N storage at a soil depth interval of 0-100 cm was 63.10 ± 27.41 Tg (Tg = 10^{12} g), with an average soil N stock of 2.44 ± 1.06 kg m^{-2} in the TRSR shrublands. Although the type of vegetation community had a small effect on soil N stock, the latter increased with increasing shrubland ground cover and soil organic carbon. However, soil N stock decreased with increasing topographical slope and MAT. Furthermore, changes in MAT primarily affected the N stock of topsoil. Among all the controlling factors, soil organic carbon explained most of the variation in the soil N stock. Considering the effects of global warming, an increase in MAT has decreased the soil N stock. Long-term monitoring of changes in soil N stock should be conducted to improve the precise estimation of soil N storage across the shrublands in the TRSR of the Tibetan Plateau.

Keywords: Soil N Storage, Ground Cover, SOC, MAT, Alpine Shrublands, Tibetan Plateau

Introduction

Nitrogen (N) is an important limiting nutrient in northern ecosystems and is widely considered to be one of the most important elements in nutrient cycles (Augusto et al. 2017). Within the N cycle in soils, significant greenhouse gases can be produced from ammonia (NH_3) volatilisation and denitrification (NO_X – Vitousek & Farrington 1997). As a key element, the storage of N in the soil is a basic input parameter for greenhouse gas, vegetation, and land surface models (Todd-Brown et al. 2014). Thus, studying soil N storage and its controlling factors is important to understand the relationship between carbon cycles in terrestrial ecosystems and global climate change (Zhao et al. 2018).

Human activities, such as fertiliser use, industrial development, burning of biomass, expansion of agriculture, and deforestation have affected the N cycle (Tian et al. 2006) and even accelerated N deposition rate (Holland et al. 2005). Several studies have shown that global N deposition was 3-5 times higher in recent years than in the last century (Janssens et al. 2010). In many regions, especially in Asia, N deposition is expected to increase faster (Chen et al. 2016). In China, over the past decades, total N deposition has not only significantly increased, but has also increased faster than that in Europe and the United States of America, even in the high-altitude region of the Tibetan Plateau (Han et al. 2019). Accurate quantification of soil N stock is important for assessing the N capacity of soils to act as N sinks. Furthermore, uncertainties exist in the estimation of soil N stocks. Globally, researchers have estimated different soil N storages at 0-100 cm depths, for example, approximately 95 Pg (Post et al. 1985) and 133-140 Pg (Batjes 1996). At a national scale, soil N storage in China (0-100 cm) was reported to be 7.4 Pg (Yang et al. 2007), which was lower than the previously estimated 8.29 Pg (Tian et al. 2006).

Soil characteristics (Augusto et al. 2017), climatic factors (Liu et al. 2017), topography (Zhang et al. 2018), and vegetation properties (Marty et al. 2017) have significant effects on soil N stock. Research has also demonstrated that soil depths, soil types (Yang et al. 2007), and soil parent materials (Augusto et al. 2017) can affect soil N concentration and the over-soil N stock. Soil organic carbon (SOC) was observed to exhibit similar spatial distribution

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to soil N in the Loess Plateau of China (Fang et al. 2019) and eastern Rio Grande Plains, Texas, USA (Zhou et al. 2018). Climatic changes have been observed to significantly affect nutrient dynamics, especially for N (LeBauer & Treseder 2008), and warmer, wetter climates contribute to soil N processes (Liu et al. 2017) including leaching and denitrification, which inevitably affect soil N storage.

Geomorphic disturbances and terrain characteristics (Obu et al. 2017) have also been demonstrated to affect soil N. Topography, including slope, has been found to play an important role in shaping microclimate environments, resulting in different temperatures and moisture levels, thus affecting the distribution of plant communities and various soil processes (Bale et al. 1998). However, topographical slope has also been found to significantly impact soil erosion (Cardinale et al. 2007), thus affecting soil N. This demonstrates that topographical factors play an important role in predicting soil N storage, and ignoring topographical effects increases the uncertainty of estimating soil N stock, especially in mountain regions (Zhang et al. 2018).

In Mediterranean regions, vegetation types, including native and reforested plants, have also been found to affect soil N storage (Lozano-García et al. 2016). However, the extensive heterogeneity found in soils has made vegetation types poor predictors of soil N stock (Tian et al. 2006). Specifically, soil N in the forest and woodlands of northern China was 14% higher than that in the wetlands. In contrast, soil N in the wetlands was found to be more than twice that in the forest and woodlands of southern China (Tian et al. 2006).

On the Tibetan Plateau, the following factors affect the soil N storage: yak grazing in the alpine meadows (Ma et al. 2016), soil characteristics, such as paedogenesis and physicochemical parameters in the alpine meadows (Mu et al. 2016), and different land cover types, including alpine wet meadows, alpine deserts, alpine steppes, alpine meadows, and barren lands (Zhao et al. 2018). However, less attention has been paid to the shrublands of the Tibetan Plateau (Nie et al. 2017). It has been demonstrated that the mean annual temperature (MAT), instead of mean annual precipitation (MAP), primarily affects the soil N stock at a soil depth interval of 0-30 cm in the alpine shrublands of the Tibetan Plateau (Nie et al. 2017). However, previous research on the effects of climatic factors on soil N stock has only focused on topsoil (0-30 cm), while the response of soil N stock to increasing MAT in deeper soils, such as at 0-100 cm, remains unknown in these shrublands. Therefore, other factors affecting soil N stock in these shrublands should be studied.

Although uncertainties in the estimation of global N stocks usually exist, regional evaluations increase the precision of estimates (Yang et al. 2007). At a regional scale, the Three Rivers Source Region (TRSR) is distributed across the interiors of the Tibetan Plateau (Luo et al. 2014). So far, soil N storage has not been estimated in the TRSR. Regarding global warming, the TRSR has not only experienced climatic changes, but the changes experienced here were also greater than those experienced in other regions (Luo et al. 2014). It has been demonstrated that MAT has increased by 1.5 °C in the last 40 years (Qin 2014), which will change the soil N stock (Marty et al. 2017). In this study, our aim was to evaluate the soil N storage across the shrublands of the TRSR and examine the factors controlling it. We tested the following hypotheses: (1) soil characteristics (such as SOC), geographic conditions (such as slope), and vegetation characteristics (including different dominant community types and ground cover of shrublands) can not only shape soil N stock, but also explain most variations in the soil N stock across the shrublands of the TRSR; (2) the effects of MAT on soil N stock are concentrated in the topsoil and soil N stock at depths of 0-100 cm is relatively insensitive to the changes in MAT.

Materials and methods

Study area

The study area was situated between latitudes 31.65 °N to 37.02 °N and longitudes 89.40 °E to 102.45 °E. The TRSR, also known as the "water tower of China" due to it consisting mainly of the Lancang (Mekong), Yellow, and Yangtze rivers, is situated in the central Tibetan Plateau which has the highest altitude, largest area, and most crowded distribution of rivers, lakes, and glaciers worldwide (Qin 2014). Hence, despite the fragile ecological environment, the TRSR performs significant functions as an ecological security barrier for the Tibetan Plateau (Luo et al. 2014). Therefore, a nature reserve was established on the TRSR in 2000. Due to its unique geographical position and rich biodiversity, a Chinese national park was established in the TRSR in 2019. This upgrade from nature reserve to national park implied stricter management in the TRSR, ensuring less human disturbance. Alpine shrublands, a significant biome in the TRSR, is dominated by woody plants, such as *Sibiraea laevigata, Potentilla fruticose, Rhododendron capitatum, and R. thymifolium*, and many herbs, including *Kobresia capillifolia, Leymus secalinus, Stipa purpurea*, and *K. parva.* The main soil types in the region are leptosols, calcisols, chernozems, and cambisols, based on the soil classification system of the Food and Agriculture Organization (FAO) of the United Nations (Nachtergaele et al. 2012).

The climate of the TRSR represents a typical continental plateau climate (Fan et al. 2010). The mean altitude is 4000 m a.s.l., while the MATs and MAPs in the study area range from -5.6 to 3.8 °C and from 262 to 773 mm, respectively (Qin 2014). Like the Tibetan Plateau, the TRSR is moving toward a warmer and wetter climate (Qin 2014). Specifically, the MAT in the TRSR has been increasing by up to 0.27-0.33 °C every decade (Qin 2014). Furthermore, MAP has also been increasing in the TRSR by up to 1.35 mm year^{-1} (Qin 2014).

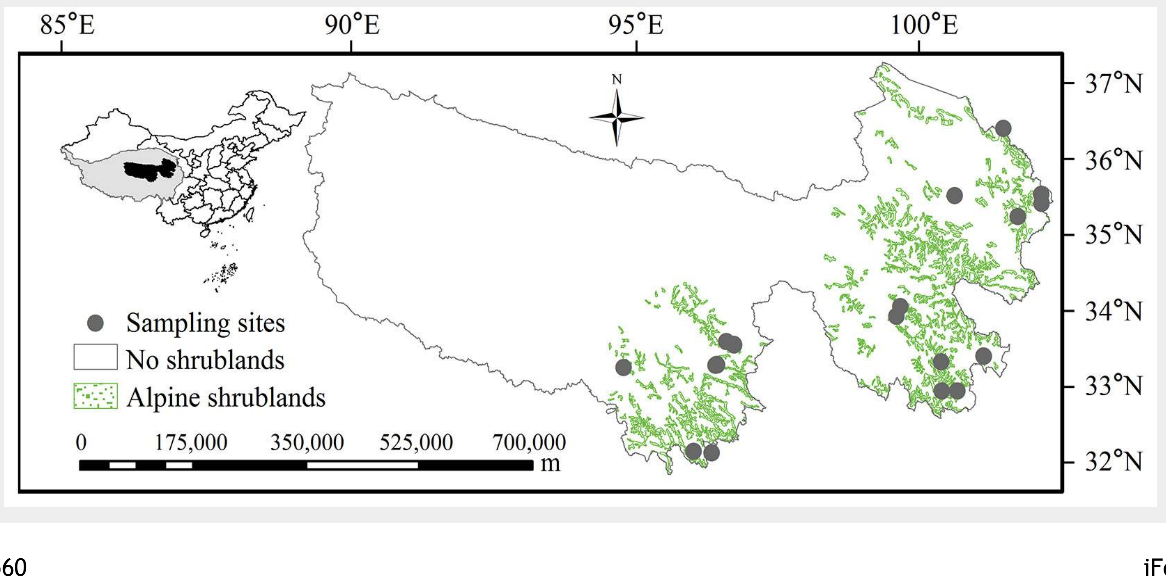

Fig. 1 - The distribution of 22 sampling sites in the shrublands of the Three Rivers Source Region shown on a 1:1,000,000 scale background vegetation map (Chinese Academy of Science 2001).

Field survey and laboratory measurements

To estimate the soil N stock in the alpine shrublands of the TRSR, a total of 66 soil profiles were systematically sampled from 22 typical sites in the shrublands from July to August from 2011 to 2013 (Fig. 1). Field surveys were conducted following the method prescribed by the Technical Manual Writing Group of the Ecosystem Carbon Sequestration Project (2015). First, we conducted a preliminary survey of potential study sites based on the 1:1,000,000 scale vegetation atlas of China (Fig. 1). Afterwards, we confirmed the representativeness of the selected sites in the field. These selected sites had to satisfy two requirements: (i) the dominant community of shrublands should be larger than 100 m²; and (ii) the site should have a relatively even distribution of habitat, species composition, and community structure (Ecosystem Carbon Sequestration Project 2015). At each site, geographical conditions, such as slope, were determined using a geological compass (DQY-1A®, Optical Instrument Company, Haerbin, China), and three 5 × 5 m plots were identified. The distance between them was never less than 5 m or further than 50 m. Primary vegetation characteristics, such as the ground cover of woody plants and dominant woody species were visually estimated and recorded in each plot. At each plot, a soil profile (1 m long × 1.5 m wide × 1 m deep) was dug for the collection of samples at different depths (0-10, 10-20, 20-30, 30-50, 50-70, and 70-100 cm). Three sub-samples were collected using a standard 100 cm³ cylindrical container (height: 50 mm; diameter: 50.5 mm) for each depth and then mixed to form one composite sample per plot. The sample was dried at 105 °C for 24 h in the lab and then gravimetrically weighed. The soil bulk density of each sampled horizon was calculated as the ratio between the dry weight and total volume. Soil samples were then sieved at 2.0 mm. SOC was measured through wet oxidation following the Walkley-Black method, and organic matter was oxidised by adding sulphuric acid to potassium dichromate. Total soil N was measured through dry combustion using an elemental analyser (2400 CHNS/O® elemental analyser, Perkin-Elmer, Waltham, MA, USA). The temperatures for combustion and reduction were set to 950 °C and 640 °C, respectively.

Climate data

To analyse the effects of climatic factors on soil N stock, the MAT data for each site were obtained from the WorldClim database (http://www.worldclim.org/), with a spatial resolution of 1 × 1 km (Hijmans et al. 2005).

Data analysis

To determine the soil N stock and storage (their results and corresponding standard deviations using the 22 sites) at each soil depth, the following formulas were used (eqn. 1, eqn. 2).

$$ND_i = \sum_{i=1}^{n} T_i N_i BD_i \cdot \frac{1-C_i}{100} \quad (1)$$

$$NS_i = ND_i \cdot Area \quad (2)$$

where *ND* is the soil N stock (kg N m⁻²), T_i is the soil thickness (cm), N_i is the total N content (g kg⁻¹), BD_i is the bulk density (g cm⁻³), and C_i is the volume percentage of the fraction > 2 mm in layer *i* (cm). In eqn. 2, NS_i is the soil N storage at layer *i* (cm), and *Area* is the area of shrublands across the TRSR.

The effects of MAT, SOC, and topographical slope of the shrublands on soil N stock were determined using ordinary least squares regression. Curve estimation was used to estimate the relationship between the ground cover of shrublands and soil N stock. Normality and homogeneity of variance were satisfied, and one-way analysis of variance (ANOVA) was used to compare the differences between various dominant community types. These analyses were performed using SPSS® v. 22.0 (IBM Corporation, Armonk, NY, USA) and the graphs were drawn using Origin® 2017 (OriginLab, Northampton, MA, USA). Variables that did not significantly contribute to the soil N stock were excluded from the variation partitioning analysis. The variation in soil N stock at depths of 0-30, 30-50, and 50-100 cm layers was partitioned using four explanatory factors that included SOC, slope, ground cover, MAT, and their combined effects. This analysis was performed using the "vegan" R software package (R Development Core Team 2012).

Tab. 1 - Stock and storage of soil nitrogen (N) from soil surface to a depth of 100 cm in the shrublands of the Three Rivers Source Region. The results of soil N storage are shown as mean values ± standard deviation (SD).

Soil depth (cm)	Soil N stock (kg m⁻²)				Soil N storage (Tg)
	Min	Max	Mean	SD	
0-10	0.14	0.79	0.40	0.17	10.43 ± 4.44
0-20	0.35	1.45	0.80	0.28	20.78 ± 7.35
0-30	0.45	1.87	1.14	0.38	29.44 ± 9.72
0-50	0.61	2.69	1.64	0.54	42.40 ± 14.02
0-70	0.61	3.58	2.02	0.75	52.22 ± 19.39
0-100	0.61	4.72	2.44	1.06	63.10 ± 27.41

Results

Storage, stock, and content of soil N in TRSR shrublands

The soil N storage in the TRSR shrublands was 63.10 ± 27.41 Tg at 0-100 cm soil depth interval, with an average soil N stock of 2.44 ± 1.06 kg m⁻² (Tab. 1). The soil N content decreased with increasing soil depth and at the soil depth intervals of 0-10, 10-20, 20-30, 30-50, 50-70, 70-100 cm, it was found to be 6.44 ± 1.83, 5.23 ± 1.25, 4.25 ± 1.44, 3.32 ± 1.47, 2.68 ± 1.54, and 2.34 ± 1.93 g kg⁻¹, respectively (Fig. 2).

Factors controlling soil N stock in TRSR shrublands

SOC significantly affected soil N stock (Fig. 3). Specifically, soil N stock increased with SOC at soil depth intervals of 0-30 cm (Fig. 3A), and similar observations were made at soil depth intervals of 0-50 (Fig.

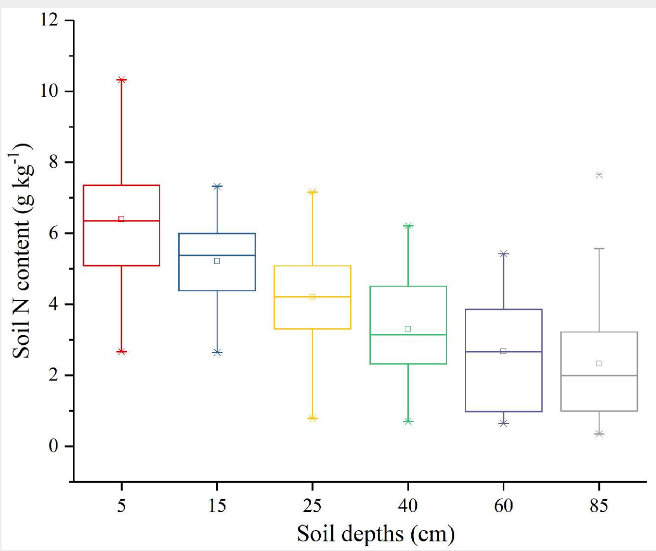

Fig. 2 - Soil nitrogen (N) content at different soil depths across the shrublands of the Three Rivers Source Region.

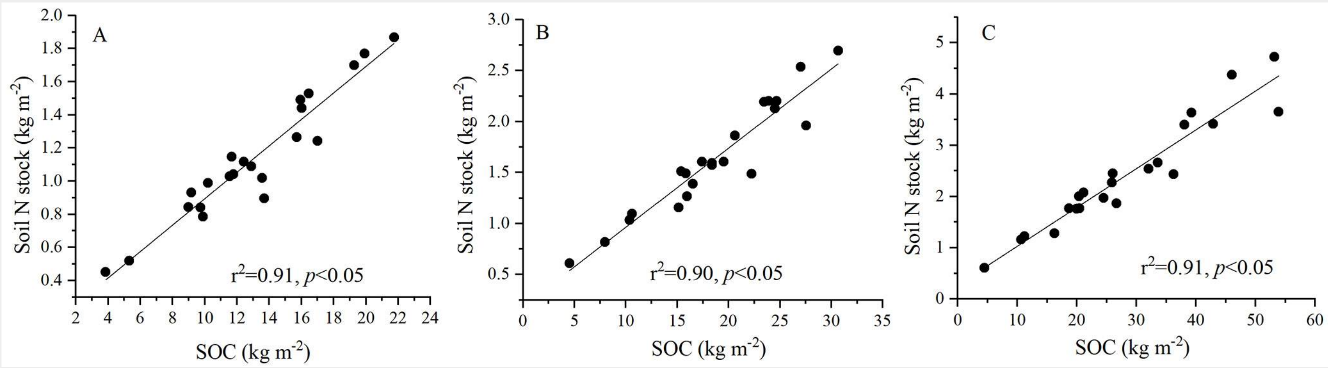

Fig. 3 - Relationship between soil organic carbon (SOC) and soil nitrogen (N) stock in the shrublands of the Three Rivers Source Region at the soil depth intervals of (A) 0-30 cm, (B) 0-50 cm, and (C) 0-100 cm.

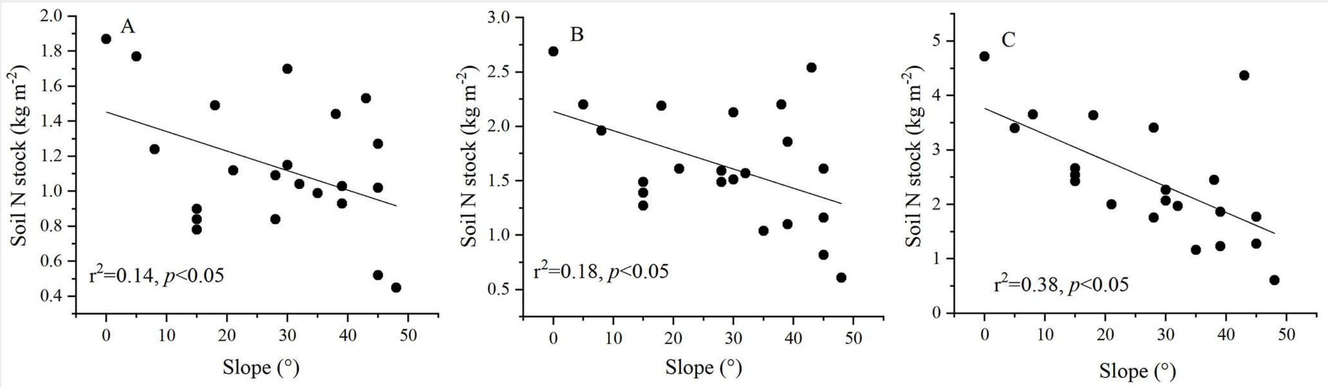

Fig. 4 - Relationship between slope and soil nitrogen (N) stock in the shrublands of the Three Rivers Source Region at the soil depth intervals of (A) 0-30 cm, (B) 0-50 cm, and (C) 0-100 cm.

3B) and 0-100 cm (Fig. 3C).

Slope was also found to significantly affect soil N stock (Fig. 4). However, contrary to the relationship between SOC and soil N density, soil N stock decreased with increasing slope at the soil depth interval of 0-30 cm (Fig. 4A), and similar observations were made at depth intervals of 0-50 (Fig. 4B) and 0-100 cm (Fig. 4C).

The ground cover of shrublands can significantly stimulate the accumulation of soil N stock; indeed, it was observed to increase with ground cover at all soil depth intervals of 0-30 (Fig. 5A), 0-50 (Fig. 5B), and 0-100 cm (Fig. 5C).

Soil N stock differences were observed for different shrubland community types, especially for *P. fruticose* and *S. laevigata* (Fig. 6). However, these differences were not significant ($p > 0.05$) according to the ANOVA results.

Soil N stock was found to significantly decrease with increasing MAT ($p < 0.05$) at soil depth intervals of 0-30 (Fig. 7A) and 0-50 cm ($p < 0.05$ – Fig. 7B). However, a decreasing trend was not observed at soil depth intervals of 0-100 cm ($p > 0.05$ – Fig. 7C), which supported the hypothesis that the effects of MAT on soil N stock were concentrated in the topsoil, with deeper soils having a small effect.

Variables, such as slope, ground cover, SOC, and MAT, can significantly affect soil N stock at soil depth intervals of 0-30 and 0-50 cm; thus, these variables were selected to conduct variation partitioning analyses. Considering that the effect of MAT on soil N stock was insignificant at the soil depth interval of 0-100 cm, it was not included in the variation partitioning analyses for this depth interval. The results showed that the soil N stock was effec-

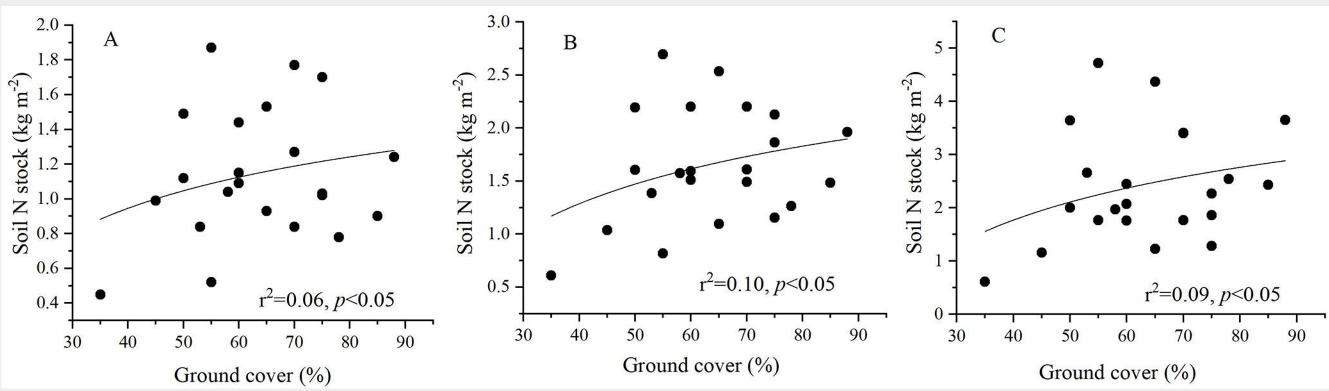

Fig. 5 - Relationship between ground cover and soil N stock in the shrublands of the Three Rivers Source Region at the soil depth intervals of (A) 0-30 cm, (B) 0-50 cm, and (C) 0-100 cm.

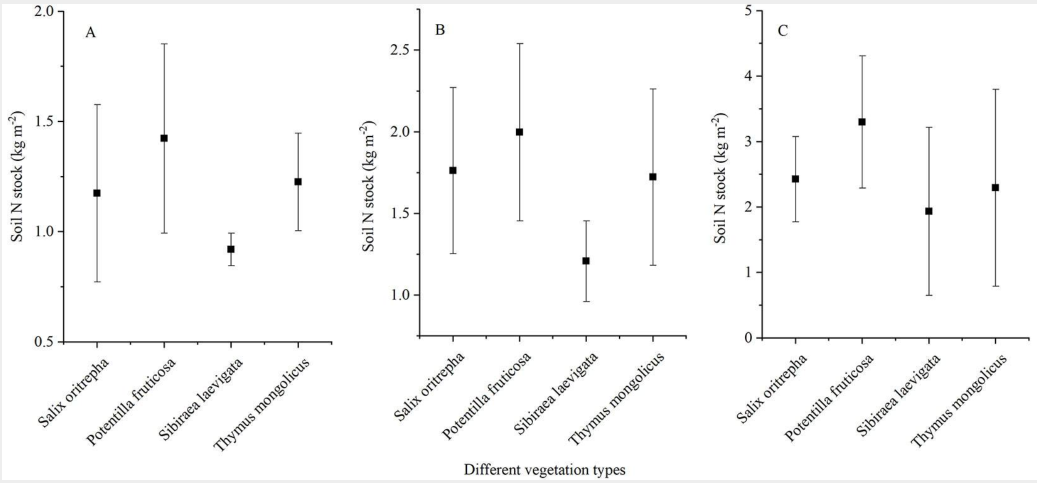

Fig. 6 - Relationship between vegetation types and soil N stock in the shrublands of the Three Rivers Source Region at the soil depth intervals of (A) 0-30 cm, (B) 0-50 cm, and (C) 0-100 cm. The solid black squares and error bars represent the mean of each measurement and corresponding standard deviations, respectively.

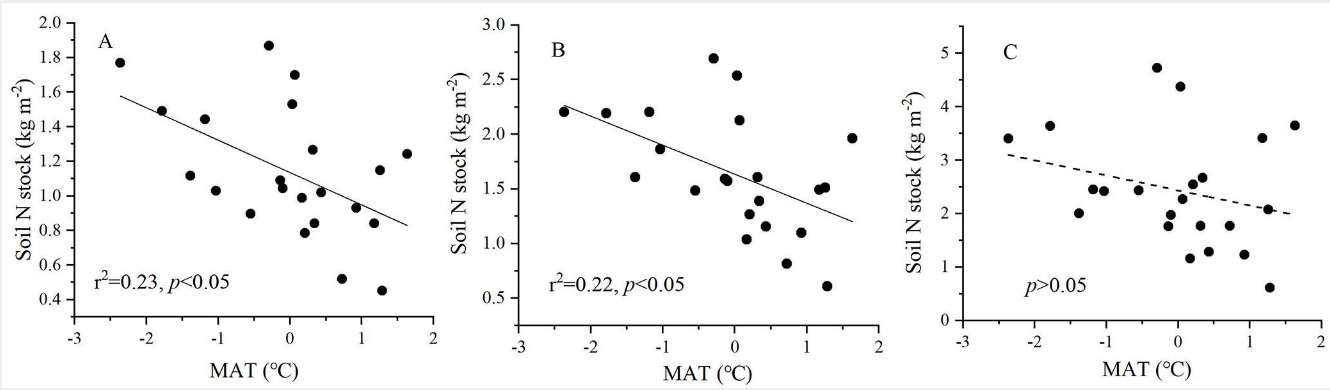

Fig. 7 - Relationships between mean annual temperature (MAT) and soil nitrogen (N) stock in the shrublands of the Three Rivers Source Region at the soil depth intervals of (A) 0-30 cm, (B) 0-50 cm, and (C) 0-100 cm.

tively explained by the selected variable, supporting the hypothesis. The amount of variation captured by all selected factors was 94.52%, 94.71%, and 94.31% for soil N stock at soil depth intervals of 0-30, 0-50, and 0-100 cm, respectively (Fig. 8). Individual SOC explained the most variations in the soil N stock for up to 68.54%, 63.94%, and 58.16% at soil depth intervals of 0-30, 0-50, and 0-100 cm, respectively (Fig. 8).

Discussion

Relationship between SOC and soil N stock

Soil N stock was observed to increase with increasing SOC at all soil depth intervals of 0-30, 0-50, and 0-100 cm across the shrublands of the TRSR (Fig. 3). Similar observations have been made for the Loess Plateau (Fang et al. 2019). It has been demonstrated that N in soil is mainly determined by the decomposition and accumulation of soil organic matter (Zhou et al. 2018, Fang et al. 2019), which corresponds with the positive correlation observed between soil N stock and SOC.

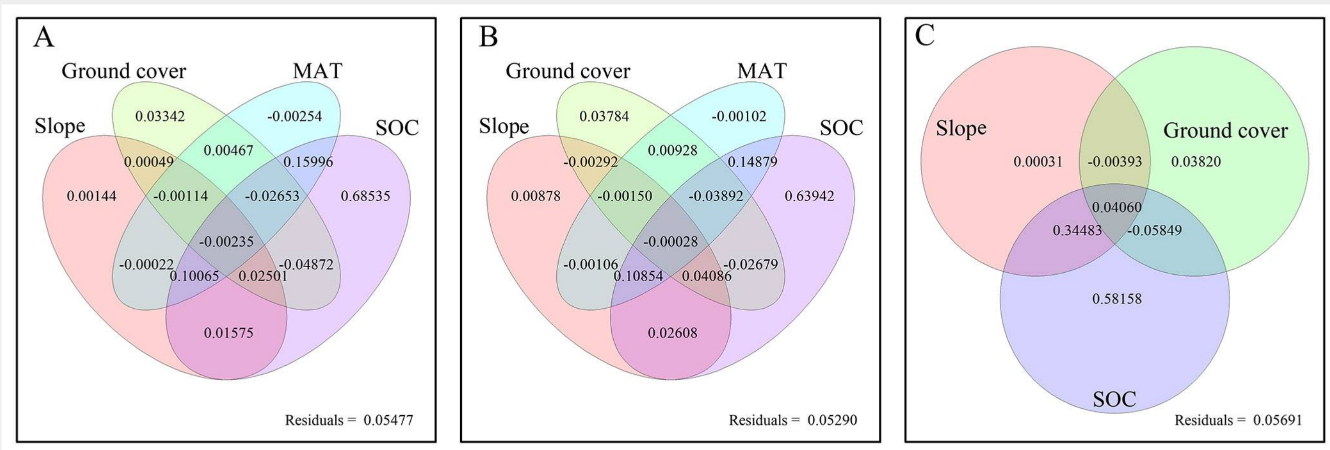

Fig. 8 - Results of variation partitioning analysis for soil nitrogen (N) stock at the soil depth intervals of in (A) 0-30 cm, (B) 0-50 cm, and (C) 0-100 cm across the shrublands of the Three Rivers Source Region. Variation partitioning analysis consists of explained variation, including effects of slope, ground cover, soil organic carbon (SOC), mean annual temperature (MAT), and their joint effects, and unexplained variations.

In the TRSR, SOC has been demonstrated to contribute to a potential sink (Nie et al. 2019). Considering that and the positive correlation observed between SOC and soil N stock, a potentially increasing soil N can be inferred in future.

Relationship between soil N stock and slope

Variations in topography can result in different microclimates, inducing different plant growth and soil properties (Lozano-García et al. 2016). It has been demonstrated that, although slope does not directly affect soil N storage in the grasslands of northern China, it can affect factors such as soil pH and bulk density, which can limit soil N storage (Zhang et al. 2018). In this study, soil N stock was found to decrease with increasing slope. A steep slope can lead to soil erosion, which can significantly damage the environment and result in the loss of finer soil particles with higher N stock (Carroll et al. 2000). Therefore, soil N stock was observed to decrease with increasing slope across the shrublands of the TRSR.

The negative relationship between soil N stock and slope indicated that conservation of soil by revegetation or reduced grazing to restrain soil erosion is necessary to maintain soil fertility (He et al. 2008).

Relationship among vegetation type, ground cover, and soil N stock

The amount of soil N storage is related to biotic processes, including decomposition of organic matter and productivity of plants (Post et al. 1985, Batjes 1996). Hence, vegetation type significantly affects soil N distribution (Yang et al. 2007). However, some studies have indicated that vegetation type is a poor predictor of soil N stock in China (Tian et al. 2006), consistent with our finding that the effects of different dominant shrubland communities on soil N stock were small in the TRSR. Similarly, in temperate and boreal forests, vegetation types have been found to have only a small impact on soil N storage (Marty et al. 2017). This difference could be due to high soil heterogeneity (Tian et al. 2006), which indicates that regional research on factors controlling soil N stock is significant for understanding terrestrial N cycles.

Higher ground cover of shrublands can stimulate the accumulation of soil N storage in the shrublands of the TRSR. Increased ground cover is derived from a growing shrubland canopy, which can stimulate more litter input to soil (Cornelissen et al. 2007), thus increasing soil N stock. It has been demonstrated that climatic changes increase the ground cover of shrublands at high latitudes (Naito & Cairns 2011) and arctic and alpine regions (Myers-Smith 2011), indicating that an increase in soil N storage may occur in the future (Marty et al. 2015).

Relationship between soil N stock and MAT

Increasing temperatures can accelerate microbial metabolism, which can contribute to gaseous losses by denitrification and volatilisation, decreasing the soil N stock (Kulkarni et al. 2015). An increase in plant growth in the shrublands of the TRSR (Nie et al. 2018) has also contributed to the uptake of nutrients by plant roots, decreasing the soil N stock (Kulkarni et al. 2015). However, it should be noted that in cold and wet regions, low temperatures generally limit plant growth (Wang et al. 2012). An increasing MAT has been demonstrated to stimulate aboveground biomass in the shrublands of the TRSR (Nie et al. 2018). Thus, more litter is transferred to the soil as one of the primary input resources (Marty et al. 2017), which aids in increasing the soil N stock. The effects of limited accumulation of N may exceed the positive effects on soil N stock, resulting in a negative relationship between MAT and soil N stock.

It has been found that soil N stock at soil depth intervals of 0-30 cm decreases with increasing MAT in the alpine shrublands of the Tibetan Plateau (Nie et al. 2017). Furthermore, this study demonstrated that soil N stock at a soil depth of 0-100 cm was independent of MAT, which showed that the soil N stock in deeper soils, such as at 50-100 cm depth, was less affected by MAT in the shrublands of the TRSR.

Amid the growing effects of global warming, MAT has been increasing by 1.5 °C in the TRSR (Qin 2014). Considering only the negative relationship between temperature and soil N stock (Fig. 7), soil N storage was found to decrease. However, N dioxides have been anthropogenically emitted and may increase further (Lü & Tian 2007). Further, global warming not only affects plant growth but also shifts plant species across the tundra ecosystem (Wang et al. 2012), changing the nutrient cycle. Therefore, long-term monitoring of changes in soil N stock is necessary to improve the precision of N storage estimation across the shrublands of the TRSR.

Controlling factors of soil N stock

The controlling factors of soil N stock in the shrublands can explain most soil N stock variables, with the unexplained variations being only 5.48%, 5.29%, and 5.69% at soil depth intervals of 0-30, 0-50, and 0-100 cm, respectively (Fig. 8), indicating that the factors controlling soil characteristics (Augusto et al. 2017), topography (Zhang et al. 2018), vegetation properties (Marty et al. 2017), and climatic factors (Liu et al. 2017) significantly affected the soil N stock. The dominant variable that determined the soil N stock was SOC across the shrublands of the TRSR. The individually explained variable of soil N stock from SOC was as high as 68.54%, 63.94%, and 58.16% at soil depth intervals of 0-30, 0-50, and 0-100 cm, respectively (Fig. 8), which indicated that SOC was a robust factor controlling the soil N stock in the shrublands of the TRSR.

Conclusions

Across the shrublands in the TRSR, total soil N storage at the soil depth interval of 0-100 cm was 63.10 ± 2 7.41 Tg and the average soil N stock was 2.44 ± 1.06 kg m^{-2}. Increasing the ground cover of shrublands stimulated soil N accumulation. However, MAT and topographical slope were negatively related to soil N stock. Furthermore, the effect of MAT on soil N stock was concentrated on the topsoil. Most soil N stock variations were explained by climatic factors, soil characteristics, topography, and vegetation type in the TRSR, with SOC explaining the largest variation. In the global climate change scenarios, long-term monitoring of changes in soil N stock is necessary to improve the estimation of soil N stock across the shrublands of the TRSR in the Tibetan Plateau.

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