



Short Communication

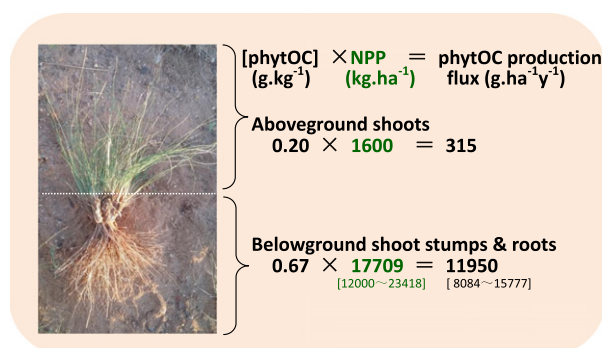
Phytolith-occluded organic carbon as a mechanism for long-term carbon sequestration in a typical steppe: The predominant role of belowground productivity

Limin Qi^a, Frank Yonghong Li^{a,*}, Zhangting Huang^b, Peikun Jiang^b, Taogetao Baoyin^a, Hailong Wang^b^a School of Ecology and Environment, Inner Mongolia University, 235 University West Road, Hohhot, Inner Mongolia 010021, China^b School of Environmental and Resource Sciences, Zhejiang A & F University, Lin'an, Zhejiang 311300, China

HIGHLIGHTS

- Occlusion of organic carbon in phytolith (phytOC) is a mechanism for long-term C sequestration.
- PhytOC content and production in the above and belowground parts of steppe plants were determined.
- PhytOC concentration was significantly higher in below than above ground plant parts.
- PhytOC production was at least one order of magnitude greater from below than above ground parts.
- Belowground part plays a dominant role in biogeochemical silica cycle and C sequestration.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 9 September 2016

Received in revised form 23 October 2016

Accepted 27 October 2016

Available online 3 November 2016

Editor: Ajit Sarmah

Keywords:

Carbon sequestration

Grassland

phytOC production

ANPP

BNPP

ABSTRACT

Phytolith-occluded organic carbon (phytOC) has recently been demonstrated to be an important terrestrial carbon (C) fraction resistant to decomposition and thus has potential for long-term C sequestration. Existing studies show that plant leaves and sheath normally have high phytOC concentration, thus most of phytOC studies are limited to the aboveground plant parts. Grassland communities comprise herbaceous species, especially grasses and sedges which have relatively high concentrations of phytoliths, but the phytOC production from grassland, especially from its belowground part, is unknown. Here we determined the phytOC concentration in different parts of major plant species in a typical steppe grassland on the Mongolian Plateau, and estimated the phytolith C sequestration potential. We found that the phytOC concentration of major steppe species was significantly ($p < 0.05$) higher in belowground (0.67 g kg^{-1}) than aboveground biomass (0.20 g kg^{-1}) and that the belowground net primary productivity (BNPP) was 8–15 times the aboveground net primary productivity (ANPP). Consequently, the phytOC stock in belowground biomass (12.50 kg ha^{-1}) was about 40 times of that in aboveground biomass (0.31 kg ha^{-1}), and phytOC production flux from BNPP ($8.1\text{--}15.8 \text{ kg ha}^{-1} \text{ yr}^{-1}$) was 25–51 times of that from ANPP. Our results indicate that BNPP plays a dominant role in the biogeochemical silica cycle and associated phytOC production in grassland ecosystems, and suggests that potential phytolith C sequestration of grasslands may be at least one order of magnitude greater than the previous estimation based on ANPP only. Our results emphasize the need for more research on phytolith and phytOC distribution and flux in both above and below ground plant parts for quantifying the phytolith C sequestration.

© 2016 Elsevier B.V. All rights reserved.

* Corresponding author.

E-mail address: lifuyong@126.com (F.Y. Li).

1. Introduction

Terrestrial biogeochemical carbon (C) sequestration is among the most promising approaches to reduce the speed of rapidly rising atmospheric carbon dioxide (CO₂) concentrations and thus mitigate impacts of climate change (IPCC, 2014; Lal, 2004). Occlusion of C within phytoliths has been recently shown to be an effective biogeochemical mechanism for long-term C sequestration (Parr et al., 2009, 2010; Parr and Sullivan, 2011; Song et al., 2012; Street-Perrott and Barker, 2008). Phytoliths are highly resistant to decomposition and phytolith-occluded organic carbon (PhytOC) may be preserved in the environment for several thousands of years after plant decomposition (Blecker et al., 2006; Parr and Sullivan, 2005; Wilding, 1967). Phytoliths are present in most plants and range in concentration from 0.5% or less in most dicotyledons, 1–3% in typical dryland grasses, and may comprise 10–15% in Cyperaceae and wetland Poaceae species (Epstein, 1994). Phytoliths contain 0.2–5.8% of PhytOC (Parr and Sullivan, 2005, 2011; Parr et al., 2010; Zhang et al., 2016; Zuo and Lü, 2011), and although the phytOC concentration in plants is low, its long residence time in soil after plant decomposition implies it an important C sink (Blecker et al., 2006). In addition, the carbon to nitrogen (C:N) ratio in phytoliths is around 41, which indicates that unlike other forms of C, phytOC does not lockup a significant amount of nitrogen (Hodson et al., 2008). Biogeochemical cycling of silica and the associated phytOC production has been increasingly studied to quantify the potential of various plant species/communities to sequester atmospheric CO₂ (Blecker et al., 2006; Conley, 2002; Parr et al., 2010; Song et al., 2012).

Grassland covers more than one fifth of the world's land surface (Scurlock and Hall, 1998), and holds nearly one-fourth of the global terrestrial C stock (Jones and Donnelly, 2005; Lal, 2004), playing a crucial role in the process of the global terrestrial C cycle. Grassland vegetation comprises mainly herbaceous species, especially Poaceae and Cyperaceae species which have been shown to have relatively high concentrations of phytoliths (Epstein, 1994; Marschner, 1995), and thus have potential for C sequestration (Blecker et al., 2006; Song et al., 2012). However, phytOC concentration in grassland species, especially in the different plant parts of different species are largely unknown. The few existing studies evaluating the potential of grasslands for phytolith C sequestration did not determine PhytOC content in phytoliths of grassland plants directly, but rather used the mean phytOC content in phytoliths and phytolith content in plants to estimate the phytOC production flux and rate; and only the aboveground part of plants was considered (Blecker et al., 2006; Song et al., 2012; Zhao et al., 2016).

PhytOC production flux in a terrestrial ecosystem is the product of plant dry matter production (i.e., net primary productivity, NPP) and the phytOC content in the dry matter. In arid and semi-arid steppe grasslands, belowground NPP (BNPP) is usually 8–15 times of the aboveground NPP (ANPP) (Chai et al., 2014; Chen and Huang, 1988; Hou et al., 2014; Ma et al., 2010). To include the belowground biomass and BNPP is essential for estimating phytOC stock and phytOC production flux in grassland ecosystems. This requires the information of plant biomass production and phytOC content in the biomass in both the above- and belowground parts of grassland plants, but this information is unavailable. Phytoliths are non-crystalline minerals in living plants through silica deposition of cell wall, fillings of cell lumen and intercellular spaces of the cortex near evaporating surfaces within plant tissue when soluble silica is absorbed by the roots (Piperno, 2006). Plant leaves and sheath normally have high phytOC concentration (Marschner, 1995). For example, a few recent studies showed that phytOC concentration was lower in roots than shoots of rice (Li et al., 2013), and is lower in culm than leaves of bamboo (Yang et al., 2015). Thus we hypothesized that grassland plants have higher phytolith and phytOC concentration in the above- than belowground parts, but we also hypothesized that BNPP plays an important role in phytolith C sequestration in grassland ecosystems, as BNPP is much greater than ANPP, even though phytOC concentration might be lower in the former.

The objective of present study was to test the above two hypotheses, that is, (1) to determine the phytOC concentration in the above- and belowground parts of the major plant species in a typical steppe grassland on the Mongolian Plateau, and (2) to estimate the phytolith C sequestration potential of the grassland, with an emphasis on quantifying the contribution of above versus below ground plant production.

2. Materials and methods

2.1. Sampling site

The study was conducted in a typical steppe grassland located around 50 km northeast of Xilinhot city, Inner Mongolia, China (latitude 44°10'N–44°12'N and longitude 116°10'E–116°12'E). The region experiences a temperate-semiarid climate, with mean annual temperature of 2.6 °C and mean annual precipitation of 267 mm. The precipitation has a large inter-annual variation from 121 to 512 mm, and 60–80% of which falls during the summer season of June to August. The soil is a sandy-loam chestnut soil (or Calcic-orthic Aridisols in the US soil taxonomy classification system). The soil has a humus layer of 20–30 cm and a calcic layer at about 40 cm in depth. The dominant vegetation is typical steppe, with dominant species *Leymus chinensis* Tzvel, *Stipa grandis* Smirn., *S. krylovii* Roshev and *Cleistogenes squarrosa* Keng.

2.2. Sampling of plant materials

Three undisturbed natural grassland sites were selected to sample plants. These three sites are located about 15 km apart but are covered with the same grassland type, dominated by *L. chinensis*, *S. grandis*, *S. krylovii*, *C. squarrosa*, *Carex korshinskyi* Kom, and *Agropyron cristatum* (L.) Gaertn. These grass and sedge species were sampled for determination of their phytolith and PhytOC content at peak plant biomass time (early September 2015). A semi-shrub species *Artemisia frigida* Willd., which is dominant in grazing-degraded grassland in the typical steppe region, was also sampled at this time.

The seven species were collected by digging up each individual plant to a depth of 20 cm below ground level at the three plots. The plant individuals were cut into three parts: aboveground parts (shoots), belowground shoot stumps (shoot stumps buried below the soil surface) and belowground roots (including rhizomes). Many plant individuals were collected at each plot to make up approximately 300 g DM (Dry Matter) for each part of each plant species. Each sample was washed three times with deionized water, dried to a constant weight at 65 °C and cut into pieces (<5 mm) for phytolith analysis.

Five quadrats of 1 × 1 m² were placed at the center and four corners of a delineated 20 × 20 m² at each plot, and all standing live and dead (that was obviously produced in current season) vascular plants in these quadrats were cut at ground level species by species, dried to a constant weight at 65 °C and weighed. The dry mass of all plant species per quadrat averaged over the five replicates was used to estimate the aboveground plant biomass at peak biomass time, and this was also used to approximate the annual ANPP of the grassland (Scurlock et al., 2002). The belowground biomass and its depth distribution (0–70 cm) during the plant growing season (May to October 2011) were measured using the soil coring method. The BNPP of grassland was calculated as the sum of increments in belowground biomass from the beginning to the end of the season and was reported by Chai et al. (2014).

2.3. Sample measurements

The phytoliths within plants were extracted with a microwave digestion process (Parr et al., 2001), followed by a Walk-Black type digestion (Walkley and Black, 1933) to ensure that extraneous organic materials in the extracted phytoliths were removed. Two duplicates were analyzed for each plant sample. The extracted phytoliths were oven-dried at 75 °C to a constant weight, and the phytolith content

calculated. The PhytOC was determined using the PhytOC alkali spectrophotometry method (Yang et al., 2014). In this method, sodium hydroxide solution was used to dissolve the silicon compound in which organic carbon is occluded, then potassium dichromate ($K_2Cr_2O_7$)-sulphuric acid (H_2SO_4) solution was used to oxidize the released organic carbon, and the concentration of Cr^{3+} produced in this oxidation was determined by spectrophotometry with its absorbance at 590 nm wavelength. The organic carbon concentration was calculated based on the amounts of potassium dichromate consumed. The accuracy and repeatability of this analytical method was well verified against the results obtained with acid dissolution-Elementar Vario MAX CN method (Germany) (Yang et al., 2014), and the method was well used in several studies (Yang et al., 2015, 2016). The phytolith and phytOC contents of different parts of each plant species were calculated as the average of the three replicate plots. For each species, the ratio of aboveground shoot mass to belowground shoot stump mass (AS/BS) was calculated based on the sampled plant individuals. The ratios were used to calculate the biomass of shoot stumps of each species per m^2 based on the measured aboveground biomass of the species. Estimation of plant root biomass mass in the entire soil profile (0–70 cm) was determined for the same grassland community by Chai et al. (2014).

2.4. Data analysis

One-way ANOVA and Duncan's Multiple Range Test ($p < 0.05$) were applied to examine the difference in phytolith and phytOC contents among different parts of plant species. Linear regression analysis was used to determine the relationship between phytolith concentration and phytOC concentration in the different plant samples. All statistics were done using SPSS 20.0. The related equations were as follows:

$$\begin{aligned} \text{Phytolith concentration (g kg}^{-1}\text{)} \\ = \text{phytolith weight (g)/dry biomass (kg)} \end{aligned} \quad (1)$$

$$\begin{aligned} \text{C concentration in phytolith (g kg}^{-1}\text{)} \\ = \text{C content in phytolith (g)/phytolith weight (kg)} \end{aligned} \quad (2)$$

$$\begin{aligned} \text{PhytOC concentration (g kg}^{-1}\text{)} \\ = \text{C content in phytolith (g)/dry biomass (kg)} \end{aligned} \quad (3)$$

$$\begin{aligned} \text{PhytOC stock (kg ha}^{-1}\text{)} \\ = \sum [\text{PhytOC concentration (g kg}^{-1}\text{)} \times \text{biomass (kg ha}^{-1}\text{)} \times 10^{-3}] \end{aligned} \quad (4)$$

$$\begin{aligned} \text{PhytOC production flux (kg ha}^{-1}\text{)} \\ = \text{PhytOC concentration (g kg}^{-1}\text{)} \times \text{NPP (kg ha}^{-1} \text{yr}^{-1}\text{)} \times 10^{-3} \end{aligned} \quad (5)$$

$$\begin{aligned} \text{PhytOC production rate (kg yr}^{-1}\text{)} \\ = \text{PhytOC production flux (kg ha}^{-1}\text{)} \times \text{area (ha)} \end{aligned} \quad (6)$$

3. Results

3.1. Phytolith concentration and PhytOC concentration in grassland plants

Both phytolith concentration and PhytOC concentration varied substantially in the aboveground shoots, belowground shoot stumps and roots (including rhizomes) among the seven species (Fig. 1A, B), with the range of 4.22–152.27 and 0.08–1.52 $g\ kg^{-1}$, respectively. There was a significant linear correlation between phytolith concentration and PhytOC concentration ($R^2 = 0.738$, $p < 0.01$) when all plant samples were included.

The bunchgrasses *S. grandis*, *S. krylovii* and *A. cristatum* had significantly ($p < 0.05$) higher phytOC concentration in belowground (buried shoot stumps, and roots and rhizomes) than aboveground plant parts, while the remaining four species had no significant difference in phytOC concentration between the above and belowground parts (Fig. 1B). PhytOC concentration in belowground parts of rhizomatous grass *L. chinensis* (mainly stem stumps and rhizomes) was lower than that in the bunchgrasses *S. grandis* and *S. krylovii* (mainly tiller stumps and roots) (Fig. 1). The mean phytolith concentration and phytOC concentration of all the seven species combined were significantly lower ($p < 0.05$) in the above - than underground parts ('Average' in Fig. 1A, B).

At the plant community level, the biomass-weighted mean phytolith concentration was significantly ($p < 0.05$) lower in aboveground ($15.75 \pm 0.51\ g\ kg^{-1}$) than belowground parts (stumps $42.24 \pm 3.49\ g\ kg^{-1}$, roots/rhizomes $42.84 \pm 8.55\ g\ kg^{-1}$). Similarly, phytOC concentration was also significantly ($p < 0.05$) lower in aboveground ($0.20 \pm 0.01\ g\ kg^{-1}$) than belowground parts (stumps $0.63 \pm 0.02\ g\ kg^{-1}$, roots/rhizomes $0.69 \pm 0.04\ g\ kg^{-1}$) (Table 1, Section 2).

3.2. PhytOC stock in different parts of grassland plants and in the community

The aboveground biomass from these undisturbed plant communities of native steppe averaged $1600\ kg\ ha^{-1}$, and the six grass and one sedge species sampled accounted for 91% of the community aboveground biomass (Table 1, Section 1). The biomass of belowground shoot stumps of each species was calculated based on the measured aboveground biomass in quadrats (gm^{-2}) and the AS/BS ratio of each sampled species; the belowground biomass of grassland community in 0–70 cm soil profile was determined by Chai et al. (2014), which was markedly greater than the aboveground biomass (Table 1, Section 1).

The phytOC stock in different parts of plant species varied greatly and ranged from 2.46 to $752.29\ kg\ ha^{-1}$ in the studied grasslands (Table 1, Section 2). The phytOC stock in aboveground biomass of the grassland community ($315\ g\ ha^{-1}$) was only about 2.52% of that in belowground biomass ($12,497\ g\ ha^{-1}$) (Table 2). As grassland BNPP was 7.5 to 14.7 times that of ANPP in the studied steppe grassland, and the biomass-weighted mean phytOC concentration of the belowground biomass ($0.67\ g\ kg^{-1}$) was significantly ($p < 0.05$) higher than that of the aboveground biomass ($0.20\ g\ kg^{-1}$), the estimated phytOC production in BNPP ($8084\text{--}15,777\ g\ kg^{-1}\ yr^{-1}$) was markedly greater than that in ANPP, comprising the dominant part (96–98%) of phytOC production in the steppe ecosystem.

4. Discussion

4.1. BNPP predominates the phytolith C sequestration in steppe ecosystems

The phytOC production flux is determined by net primary productivity (NPP) and phytOC concentration in the NPP. Our results show that biomass-weighted mean phytOC concentration in belowground biomass is significantly higher than in aboveground biomass. The BNPP is 7.5 to 14.7 times of ANPP in arid and semi-arid steppe grassland, including desert steppe, typical steppe and meadow steppe grassland (Chai et al., 2014; Chen and Huang, 1988; Hou et al., 2014; Ma et al., 2010). The higher phytOC concentration, along with the greater NPP in the below than the aboveground plant parts, makes the phytOC production from BNPP the majority (96–98%) of phytOC production in the steppe grassland. Our estimated aboveground phytOC production from the typical steppe grassland is $0.31\ kg\ ha^{-1}\ yr^{-1}$ (Table 2), which is comparable to an earlier estimate of $0.57\ kg\ ha^{-1}\ yr^{-1}$ by Song et al. (2012), considering the difference in plant species composition and ANPP in the two different studies. However, our results also show that the aboveground phytOC production is only a small part (2–4%) of total phytOC production in steppe ecosystems, suggesting that phytOC

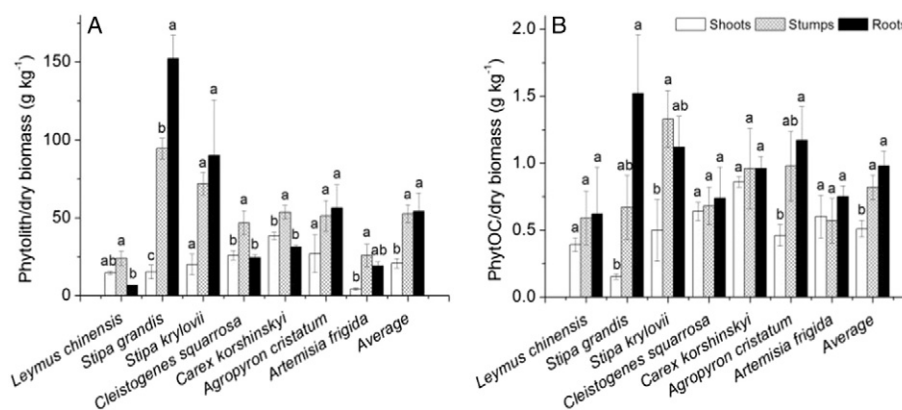


Fig. 1. Phytolith concentration (A: phytolith/dry biomass) and phytolith-occluded organic carbon concentration (B: phytOC/dry biomass) in different parts of major plant species of a typical steppe on the Mongolian Plateau. Shoots - the aboveground shoots; Stumps - the shoot stumps buried below the soil surface; Roots - including rhizomes; Average - mean of seven species.

production of grasslands calculated based only on the ANPP (e.g., in Blecker et al., 2006; Song et al., 2012) cannot represent the phytolith C sequestration potential of the grassland. The previous estimated aboveground phytOC production rates from the grassland of China, North America, or the world (Blecker et al., 2006; Song et al., 2012) may be at least one order of magnitude less than the total phytOC production rates from these grasslands. For example, the area of typical steppe in China is estimated to be $38.5 \times 10^4 \text{ km}^2$ (Song et al., 2012), thus we estimate that the annual PhytOC production from above and belowground plant parts of the typical steppe grassland in China are $1.21 \times 10^4 \text{ t yr}^{-1}$ and $31.14\text{--}60.74 \times 10^4 \text{ t yr}^{-1}$, respectively (Table 2). Furthermore, in the studies of biogeochemical cycling of silica, the ratio of soil phytolith pool (kg Si ha^{-1})/annual litterfall Si input ($\text{kg Si ha}^{-1} \text{ yr}^{-1}$) was used to estimate the residence time of soil phytoliths (Blecker et al., 2006). Our results suggest that the residence time of phytoliths in soil can be largely overestimated when this ratio was used in the estimation and the litters from plant roots (BNPP) are not included. It is vitally important to incorporate the role of BNPP in the study of the biogeochemical silica cycle and evaluation of phytolith C sequestration potential in permanent grasslands.

4.2. Large variation in phytOC concentration among different parts of grassland plants

Our results show that phytOC concentration varies substantially among different parts of the same plant species, in addition to that among species. This finding justifies the necessity to determine phytOC

concentration of different plant parts for accurate estimation of phytolith C sequestration. Our finding that phytOC concentration was significantly lower in the above- than belowground biomass is contrary to our hypothesis that phytOC concentration should be higher in the above- than belowground biomass. The mechanism that lead to this distribution pattern of phytOC concentration among plant parts are unknown. We speculate that the perennial characteristics of the grasses and sedges in the grasslands might be responsible for this pattern. The turnover rate of the belowground biomass (BGB), i.e., BNPP/mean BGB, across the season, was estimated 0.87 ($= 12,497/18,652$ in Table 2) for the studied grassland, which implies that most of the belowground biomass was produced within one plant growing season. However, this could not exclude that the part of sampled belowground biomass is perennial, thus the phytOC concentration in this part of biomass is a result of phytOC accumulation in multiple years; whereas the phytOC in aboveground biomass is completely accumulated during one plant growing season. The pattern of phytOC concentration and turnover rates of plant roots of different orders warrant further studies for accurate estimation of phytOC production.

4.3. The mechanisms of carbon occlusion within phytoliths of grassland plants

Many studies suggested that PhytOC content in plants is determined by the nature of silica deposition, the efficiency of carbon occlusion within phytoliths during phytolith formation (Parr et al., 2009, 2010; Parr and Sullivan, 2011; Zuo and Lü, 2011), and the amount of silica

Table 1
The biomass, phytOC concentration and phytOC stock of the above- and belowground parts of native steppe grassland plants on the Mongolian Plateau. The belowground part includes the plant shoot stumps buried in soil below the ground surface and roots (including rhizomes).

Plant species	<i>Leymus chinensis</i>	<i>Stipa grandis</i>	<i>Stipa krylovii</i>	<i>Cleistogenes squarrosa</i>	<i>Carex korshinskyi</i>	<i>Agropyron cristatum</i>	6 species ^a	Community
1. Biomass (kg ha^{-1})								
Aboveground shoots	849.81	307.11	130.69	130.89	7.39	40.94	1466.85	1600.20
Belowground shoot stumps	173.83	167.86	48.60	109.83	3.03	8.49	511.63	558.14
Belowground biomass ^b								18,652.20
2. PhytOC concentration (g kg^{-1})								
Aboveground shoots	0.08	0.15	0.50	0.64	0.86	0.46	0.20	0.20
Belowground shoot stumps	0.34	0.67	1.33	0.68	0.96	0.98	0.63	0.63
roots (0–20 cm)	0.61	1.52	1.12	0.74	0.96	1.17	0.69	0.69
Belowground biomass ^b								0.67
3. PhytOC stock (g ha^{-1})								
Aboveground shoots	67.98	46.07	65.35	83.77	6.36	18.83	288.35	314.57
belowground shoot stumps	59.10	112.47	64.63	74.68	2.91	8.32	322.11	351.39
Belowground biomass ^b								12,496.97

^a The determined phytOC concentration of *Artemisia frigida* was 0.63, 0.57 and 0.75 g kg^{-1} in aboveground shoots, belowground shoot stumps, and roots respectively. The species was not included in this analysis as its aboveground biomass is $<0.1\%$ in the studied native steppe communities.

^b Belowground biomass in 0–70 cm soil profile including the shoot stumps, determined by Chai et al. (2014); its phytOC concentration is the mass-weighted mean of phytOC concentration in belowground shoot stumps and roots.

Table 2

Estimated above- and belowground biomass, NPP, and phytOC production flux and rate of typical steppe ecosystems in northern China.

	Above-ground	Below-ground	Total	Below/above
Biomass (kg ha ⁻¹)	1600	18,652	20,252	11.7
NPP (kg ha ⁻¹ yr ⁻¹) ^a	1600	12,000–23,418	13,600–25,018	7.5–14.6
PhytOC concentration (g kg ⁻¹)	0.20	0.67	–	3.4
PhytOC stock (g ha ⁻¹)	315	12,497	12,812	4.8
PhytOC production flux (g ha ⁻¹ yr ⁻¹)	315	8084–15,777	8399–16,091	25.7–50.1
PhytOC production rate (× 10 ⁴ t yr ⁻¹) ^b	1.2	31.1–60.7	32.3–61.9	25.7–50.1

^a The range of belowground NPP is estimated based on the BNPP/ANPP determined in several studies on the Mongolian steppes (Chai et al., 2014; Chen and Huang, 1988; Hou et al., 2014; Ma et al., 2010) and the measurement of ANPP in this experiment.

^b The area of typical steppe in northern China is estimated 38.5 × 10⁴ km² (Song et al., 2012).

taken up by plants (Li et al., 2013). The positive correlation between phytolith content and PhytOC content in all plant samples detected in this study suggests that factors influencing the silica uptake affect phytolith and PhytOC content in grassland biomass. This result supports the early findings that grassland management enhances phytolith accumulation, e.g., by supplying nitrogen and silicon fertilizer (Guo et al., 2015; Zhao et al., 2016). More studies are needed on the factors that control the variation in both the phytoliths content and PhytOC content among different species or different parts of same species.

4.4. Conclusions

Our results represent the first study of the phytOC concentration in the different parts of plant species in natural grasslands, and the first estimation of phytolith C sequestration potential of grassland taking into account both above and belowground plant biomass. Our findings that phytOC concentration of steppe plants is higher in BNPP than ANPP, coupled with the fact that BNPP is one order of magnitude greater than ANPP, suggest the predominance of BNPP in phytolith C sequestration in grassland ecosystems. Further investigation into the biogeochemical silica cycle and associated phytolith C sequestration in permanent grasslands will need to include the contribution of belowground as well as above ground biomass.

Acknowledgement

The work was supported by National Natural Science Foundation of China (31670454) and the Ministry of Science and Technology of China (2015BAC02B04). The authors thank Dr. Coby Hoogendoorn for comments and improving the readability of the manuscript.

References

- Blecker, S.W., McCulley, R.L., Chadwick, O.A., Kelly, E.F., 2006. Biologic cycling of silica across a grassland bioclimate sequence. *Glob. Biogeochem. Cycles* 20, 4253–4274.
- Chai, X., Liang, C.Z., Liang, M.W., Han, W.H., Li, Z.Y., Miao, B.L., Wang, W., Wang, L.X., 2014. Seasonal dynamics of belowground biomass and productivity and potential of carbon sequestration in meadow steppe and typical steppe in Inner Mongolia, China. *Acta Ecol. Sin.* 34, 5530–5540 (in Chinese with abstract in English).
- Chen, Z.Z., Huang, D., 1988. The turnover rate of belowground biomass and belowground primary productivity of *Leymus chinensis* and *Stipa grandis* steppe in Xilin River Basin, Inner Mongolia. *Grassland Ecosystem Research 2*. Science Press, Beijing, pp. 132–138 (in Chinese with abstract in English).
- Conley, D.J., 2002. Terrestrial ecosystems and the global biogeochemical silica cycle. *Glob. Biogeochem. Cycles* 16, 681–688.
- Epstein, E., 1994. The anomaly of silicon in plant biology. *Proc. Natl. Acad. Sci. U. S. A.* 91, 11–17.
- Guo, F., Song, Z., Sullivan, L., Wang, H., Liu, X., Wang, X., Li, Z., Zhao, Y., 2015. Enhancing phytolith carbon sequestration in rice ecosystems through basalt powder amendment. *Sci. Bull.* 60, 591–597.
- Hodson, M.J., Parker, A.G., Leng, M.J., Sloane, H.J., 2008. Silicon, oxygen and carbon isotope composition of wheat (*Triticum aestivum* L.) phytoliths: implications for palaeoecology and archaeology. *J. Quat. Sci.* 23, 331–339.
- Hou, X., Ji, L., Wang, Z., 2014. Response of net primary productivity and C accumulation of plant communities in desert steppe and typical steppe to land use types during two hydrologically contrasting growing seasons. *Acta Ecol. Sin.* 34, 6256–6264 (in Chinese with abstract in English).
- IPCC, 2014. In: Edenhofer, O., et al. (Eds.), *Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Jones, M.B., Donnelly, A., 2005. Carbon sequestration in temperate grassland ecosystems and the influence of management, climate and elevated CO₂. *New Phytol.* 164, 423–439.
- Lal, R., 2004. Soil carbon sequestration impacts on global climate change and food security. *Science* 304, 1623–1627.
- Li, Z., Song, Z., Parr, J.F., Wang, H., 2013. Occluded C in rice phytoliths: implications to biogeochemical carbon sequestration. *Plant Soil* 370, 1–9.
- Ma, W., Fang, J.Y., Yang, Y.H., Mohammad, A., 2010. Biomass carbon stocks and their changes in northern China's grasslands during 1982 to 2006. *Sci. China Life Sci.* 53, 841–850.
- Marschner, H., 1995. *Mineral Nutrition of Higher Plants*. second ed. Academic Press, London, UK.
- Parr, J.F., Sullivan, L.A., 2005. Soil carbon sequestration in phytoliths. *Soil Biol. Biochem.* 37, 117–124.
- Parr, J.F., Sullivan, L.A., 2011. Phytolith occluded carbon and silica variability in wheat cultivars. *Plant Soil* 342, 165–171.
- Parr, J.F., Dolic, V., Lancaster, G., Boyd, W.E., 2001. A microwave digestion method for the extraction of phytoliths from herbarium specimens. *Rev. Palaeobot. Palynol.* 116, 203–212.
- Parr, J., Sullivan, L., Quirk, R., 2009. Sugarcane phytoliths: encapsulation and sequestration of a long-lived carbon fraction. *Sugar Tech.* 11, 17–21.
- Parr, J., Sullivan, L., Chen, B., Ye, G., Zheng, W., 2010. Carbon bio-sequestration within the phytoliths of economic bamboo species. *Glob. Chang. Biol.* 16, 2661–2667.
- Piperno, D.R., 2006. *Phytoliths: A Comprehensive Guide for Archaeologists and Paleoecologists*. AltaMira, New York.
- Scurlock, J.M.O., Hall, D.O., 1998. The global carbon sink: a grassland perspective. *Glob. Chang. Biol.* 4, 229–233.
- Scurlock, J.M.O., Johnson, K., Olson, R.J., 2002. Estimating net primary productivity from grassland biomass dynamics measurements. *Glob. Chang. Biol.* 8, 736–753.
- Song, Z., Liu, H., Si, Y., Yin, Y., 2012. The production of phytoliths in China's grasslands: implications to the biogeochemical sequestration of atmospheric CO₂. *Glob. Chang. Biol.* 18, 3647–3653.
- Street-Perrott, F.A., Barker, P.A., 2008. Biogenic silica: a neglected component of the coupled global continental biogeochemical cycles of carbon and silicon. *Earth Surf. Process. Landf.* 33, 1436–1457.
- Walkley, A.J., Black, I.A., 1933. An examination of degradation method for determining soil organic matter: a proposed modification of the chromic acid titration method. *Soil Sci.* 37, 29–38.
- Wilding, L.P., 1967. Radiocarbon dating of biogenic opal. *Science* 156, 66–67.
- Yang, J., Li, Y., Huang, Z., Jiang, P., Xiang, T., Ying, Y., 2014. Determination of phytolith-occluded carbon content using alkali dissolution-spectrophotometry. *Chin. J. Anal. Chem.* 42, 1389–1390.
- Yang, J., Wu, J., Jiang, P., Xu, Q., Zhao, P., He, S., 2015. A study of phytolith-occluded carbon stock in monopodial bamboo in China. *Sci. Report.* 5:13292. <http://dx.doi.org/10.1038/srep13292>.
- Yang, J., Wu, J., Jiang, P., Zhao, P., 2016. Study on phytolith-occluded organic carbon and silicon in a *Pleioblastus amarus* forest. *J. Nat. Resour.* 31, 299–308.
- Zhang, X., Song, Z., McGrouther, K., Li, J., Li, Z., Ru, N., Wang, H., 2016. The impact of different forest types on phytolith-occluded carbon accumulation in subtropical forest soils. *J. Soils Sediments* 16, 461–466.
- Zhao, Y., Song, Z., Xu, X., Liu, H., Wu, X., Li, Z., Guo, F., Pan, W., 2016. Nitrogen application increases phytolith carbon sequestration in degraded grasslands of North China. *Ecol. Res.* 31, 117–123.
- Zuo, X.X., Lü, H.Y., 2011. Carbon sequestration within millet phytoliths from dry-farming of crops in China. *Chin. Sci. Bull.* 56, 3451–3456.