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Carbon storage in Chinese grassland ecosystems: Influence of different integrative methods

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The accurate estimate of grassland carbon (C) is affected by many factors at the large scale. Here, we used six methods (three spatial interpolation methods and three grassland classification methods) to estimate C storage of Chinese grasslands based on published data from 2004 to 2014, and assessed the uncertainty resulting from different integrative methods. The uncertainty (coefficient of variation, CV, %) of grassland C storage was approximately 4.8% for the six methods tested, which was mainly determined by soil C storage. C density and C storage to the soil layer depth of 100 cm were estimated to be $8.46 \pm 0.41 \text{ kg C m}^{-2}$ and $30.98 \pm 1.25 \text{ Pg C}$, respectively. Ecosystem C storage was composed of 0.23 ± 0.01 (0.7%) above-ground biomass, 1.38 ± 0.14 (4.5%) below-ground biomass, and 29.37 ± 1.2 (94.8%) Pg C in the 0–100 cm soil layer. Carbon storage calculated by the grassland classification methods (18 grassland types) was closer to the mean value than those calculated by the spatial interpolation methods. Differences in integrative methods may partially explain the high uncertainty in C storage estimates in different studies. This first evaluation demonstrates the importance of multi-methodological approaches to accurately estimate C storage in large-scale terrestrial ecosystems.

Grasslands store approximately 10% of the organic carbon (C) in global terrestrial ecosystems¹. It has been estimated that C storage in Chinese grasslands accounts for 8% of C storage in global grasslands² and 16.7% of C storage in Chinese terrestrial ecosystems³. Some studies have demonstrated that Chinese grasslands have tremendous potential for increasing C storage through improved grassland use or management^{4–8}. Enhancing grassland C storage is currently considered one of the most effective and economical approaches to partially sequester anthropogenic CO₂ emissions in China^{9–11}. Therefore, how to accurately assess C storage in Chinese grasslands is of concern for both scientists and policymakers.

Some studies have estimated C storage of different components in Chinese grasslands using various data source. Fang *et al.*¹⁰ and Ni¹² estimated the C storage of above-ground biomass (AGB) based on Grassland Resource Survey data. Yang *et al.*¹³ estimated AGB in northern China's grasslands by field-investigated data. Some other researchers estimated the C storage of below-ground biomass (BGB) using empirical data based on root to shoot ratios (R/S)^{3,14,15}. However, current estimates of C storage in different grassland components are subject to high variation. For instance, estimates of vegetation C density range from 0.22 kg C m^{-2} to 1.15 kg C m^{-2} , and estimates of C storage range from 0.56 Pg C^{16} to 4.66 Pg C^2 in vegetation. Similarly, estimates of soil organic C (SOC) storage range from 16.70 Pg C^{16} to 53.72 Pg C^2 . The large difference among these estimates may be resulted from the difference in data used for the estimation. In addition, the application of the different methods would be another factor affecting the estimates.

Different methods have been used to estimate C storage in the different components of Chinese grasslands, including remote sensing¹⁴, modeling¹⁶, and field investigation data¹³. However, soil C estimate was insufficient in Chinese grasslands because the remote sensing method was not appropriate to estimate it. Based on this, field-investigated data were always used to estimate C storage, especially soil C, at the national scale by the mean C density method. Considering the field-investigate data was scarce, the data has been integrated according to grassland type¹², climate zone¹³, administrative region¹⁴, and spatial interpolation¹⁷. Therefore, differences in the various scale-up methods maybe have primarily contributed to the inconsistency of results among different studies. To the best of our knowledge, no study has attempted to investigate how different integrative methods

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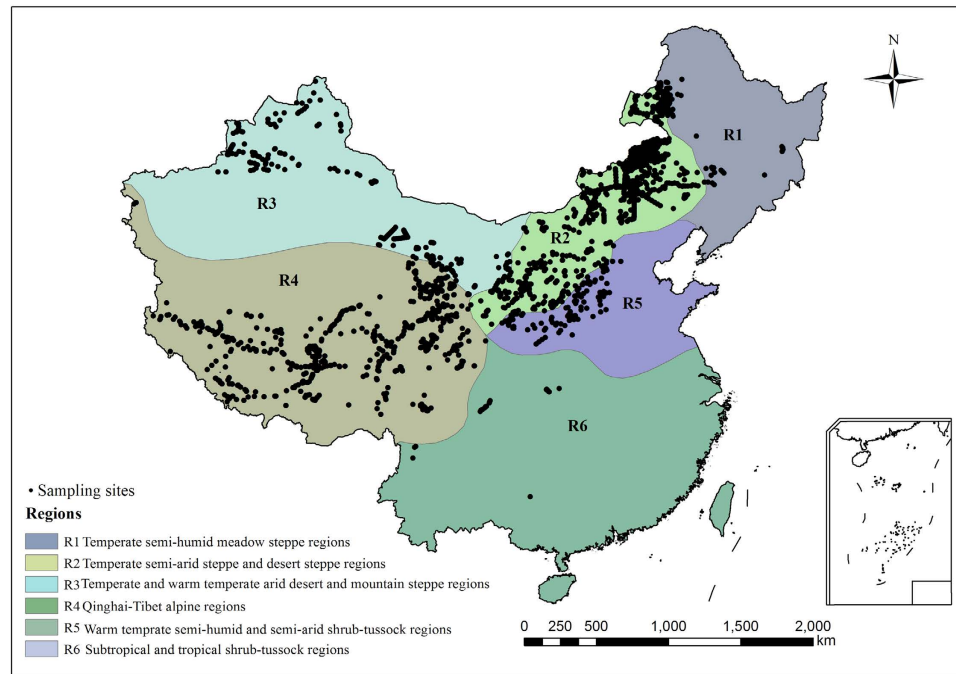


Figure 1. Locations of the sampling sites and ecological regions. The figure was created using Arcgis 8.2 software (ESRI Inc., Redlands, CA).

influence the estimation of C storage of all main components (AGB, BGB, and SOC) in Chinese grasslands using field-measured data at the national scale and the associated uncertainty.

In this study, we collected field-measured data from papers published from 2004 to 2014, including AGB, BGB, and SOC, and used six integrative methods (three spatial interpolation methods and three grassland classification methods) to calculate the C storage of different components in Chinese grasslands. We have addressed two fundamental questions in this paper: (1) To investigate the uncertainty of the estimates of C density and storage in grasslands resulting from the selected integrative methods, and (2) To evaluate C storage in all components of Chinese grasslands (including AGB, BGB, and SOC) on the basis of different integrative methods at the national scale.

Results

Carbon density and storage based on spatial interpolation methods. The uncertainty (coefficient of variation [CV], %) of C density estimated by three spatial interpolation methods was different among six grassland regions (Fig. 1) and different components (Fig. 2). The range of AGBC was $0.018 \sim 0.312 \text{ kg C m}^{-2}$, and the highest and lowest CVs for AGBC were in R1 (13.1%) and R5 (1.3%). The range of BGBC was $0.061 \sim 1.314 \text{ kg C m}^{-2}$, and the highest and the lowest CVs for BGBC were in R6 (22.9%) and R1 (4.1%). For SOC, R1 and R3 had higher CVs, which were 10.5% and 7.3%, respectively. The range of SOC was $1.892 \sim 24.856 \text{ kg C m}^{-2}$, and the CV value was about 5.8%. Overall, the CV values of C density were highest in BGBC, followed by SOC and AGBC (Fig. 2).

Furthermore, the CVs of grassland C density differed among regions, with a mean CV of approximately 5.5% (Fig. 2). The CV value of grassland C density was close to the variation of SOC density, because 94.8% of C was found to be stored in the soils of Chinese grasslands.

The C density of different components in Chinese grasslands differed significantly in different regions ($F = 62.10, P < 0.01$ for AGBC; $F = 11.46, P < 0.01$ for BGBC; $F = 13.25, P < 0.01$ for SOC; $F = 11.99, P < 0.01$ for grassland C density; Table 1). The C density of Chinese grasslands at the national scale was estimated as $8.66 \pm 0.50 \text{ kg C m}^{-2}$ using three spatial interpolation methods, which estimated AGBC, BGBC, and SOC as $0.08 \pm 0.01, 0.42 \pm 0.03, \text{ and } 8.16 \pm 0.48 \text{ kg C m}^{-2}$, respectively (Fig. 3). Overall, the total C storage in Chinese grasslands (including AGB, BGB, and SOC in the 0–100 cm soil layer) was $31.49 \pm 1.74 \text{ Pg C}$.

Carbon density and storage estimates using grassland classification methods. The CV values resulting from different grassland classification methods were different for the different components in Chinese grasslands (Fig. 4). The CV value for AGBC exceeded 10% in all grassland types, and the CV value of all grasslands was 7.2%, which ranged 0.021 to $0.342 \text{ kg C m}^{-2}$. The CV of BGBC for all grasslands 9.8%, with higher values found in steppe (29.8%) and in desert (28.2%), and the range of BGBC was $0.041 \sim 1.844 \text{ kg C m}^{-2}$. The CV value of SOC in all grassland types was 5.2%, with the highest CV in shrub-tussock (13.9%), and the range of SOC was $2.197 \sim 21.850 \text{ kg C m}^{-2}$. The CV values of grassland C density ranged from 5.2% to 13.0% across all grassland types, with a mean CV of 2.2%.

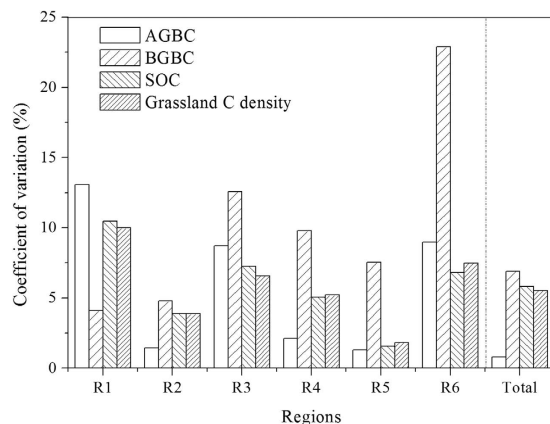


Figure 2. Coefficient of variation (CV, %) of carbon density for three spatial interpolation methods applied to different components and regions. AGBC, carbon density of the above-ground biomass; BGBC, carbon density of the below-ground biomass; SOC, SOC density. R1, temperate semi-humid meadow steppe regions; R2, temperate semi-arid steppe and desert steppe regions; R3, temperate and warm temperate arid desert and mountain steppe regions; R4, Qinghai-Tibet alpine regions; R5, warm temperate semi-humid and semi-arid shrub-tussock regions; R6, subtropical and tropical shrub-tussock regions.

| Region | Area (10 ⁶ ha) | C density (kg C m ⁻²) | | | | C storage (Pg C) | | | | CV (%) |
|-----------------|---------------------------|-----------------------------------|---------------------|---------------------|--------------------|------------------|---------|---------|-----------|--------|
| | | AGBC [§] | BGBC | SOC | Ecosystem | AGBC | BGBC | SOC | Ecosystem | |
| R1 [‡] | 19.2 | 0.094 ^{††} | 0.369 ^{ab} | 9.359 ^a | 9.823 ^a | 0.018 | 0.071 | 1.794 | 1.883 | 10.0 |
| | | (0.012) | (0.015) | (0.979) | (0.981) | (0.002) | (0.003) | (0.188) | (0.188) | |
| R2 | 63.6 | 0.059 ^b | 0.345 ^{ab} | 6.944 ^{bc} | 7.348 ^b | 0.037 | 0.219 | 4.415 | 4.672 | 3.9 |
| | | (0.001) | (0.016) | (0.268) | (0.284) | (0.001) | (0.010) | (0.170) | (0.180) | |
| R3 | 76.8 | 0.062 ^b | 0.285 ^a | 7.792 ^b | 8.139 ^b | 0.048 | 0.219 | 5.984 | 6.250 | 6.6 |
| | | (0.005) | (0.036) | (0.565) | (0.535) | (0.004) | (0.028) | (0.434) | (0.411) | |
| R4 | 153.8 | 0.046 ^c | 0.416 ^{ab} | 9.221 ^a | 9.683 ^a | 0.071 | 0.640 | 14.179 | 14.890 | 5.2 |
| | | (0.001) | (0.041) | (0.465) | (0.505) | (0.002) | (0.063) | (0.715) | (0.776) | |
| R5 | 9.7 | 0.121 ^d | 0.580 ^b | 6.649 ^c | 7.351 ^b | 0.012 | 0.056 | 0.647 | 0.716 | 1.8 |
| | | (0.002) | (0.044) | (0.103) | (0.135) | (0.001) | (0.004) | (0.010) | (0.013) | |
| R6 | 32.0 | 0.117 ^d | 0.511 ^{ab} | 9.012 ^a | 9.639 ^a | 0.037 | 0.163 | 2.882 | 3.083 | 7.5 |
| | | (0.010) | (0.117) | (0.614) | (0.721) | (0.003) | (0.037) | (0.196) | (0.231) | |
| Total | 355.05 | 0.083 | 0.418 | 8.163 | 8.664 | 0.223 | 1.369 | 29.902 | 31.494 | 5.5 |
| | | (0.001) | (0.029) | (0.475) | (0.504) | (0.001) | (0.085) | (1.654) | (1.74) | |

Table 1. Regional distribution and variation in carbon density and storage of Chinese grasslands using three spatial interpolation methods. [†]Data represent the mean and standard deviation (parentheses); data with the same lower letter in the same column indicates no significant differences at the $P = 0.05$ level. [‡]R1, temperate semi-humid meadow steppe regions; R2, temperate semi-arid steppe and desert steppe regions; R3, temperate and warm temperate arid desert and mountain steppe regions; R4, Qinghai-Tibet alpine regions; R5, warm temperate semi-humid and semi-arid shrub-tussock regions; R6, subtropical and tropical shrub-tussock regions (seeing Fig. 1). [§]AGBC, carbon density of the above-ground biomass; BGBC, carbon density of the below-ground biomass.

The C density differed significantly in the different components of various grassland types ($F = 29.90$, $P < 0.01$ for AGBC; $F = 60.49$, $P < 0.01$ for BGBC, $F = 46.32$, $P < 0.01$ for SOC, and $F = 50.55$, $P < 0.01$ for grassland; Table 2). The mean C densities for AGB, BGB, SOC, and grassland were 0.08 ± 0.01 , 0.41 ± 0.04 , 7.76 ± 0.16 , and 8.25 ± 0.18 kg C m⁻², respectively. Correspondingly, total C storage was estimated as 30.47 ± 0.28 Pg C for Chinese grasslands (down to 100 cm soil layer), with AGB, BGB, and SOC containing 0.24 ± 0.02 , 1.39 ± 0.21 , and 28.84 ± 0.14 Pg C, respectively.

Carbon storage in Chinese grasslands and uncertainty. Figure 5 provides the C density estimates for different components in Chinese grasslands based on six integrative methods. The mean C densities of AGB, BGB, and SOC were 0.08 ± 0.01 , 0.42 ± 0.03 , and 7.96 ± 0.39 kg C m⁻², respectively, at the national scale. Overall, the C density of BGB had the highest CV (7.6%) among all components. However, the CV value of grassland C density was close to that of SOC, where they were 4.8% and 4.9%, respectively. The C density of Chinese grasslands was 8.46 ± 0.41 kg C m⁻², most of which stored as SOC.

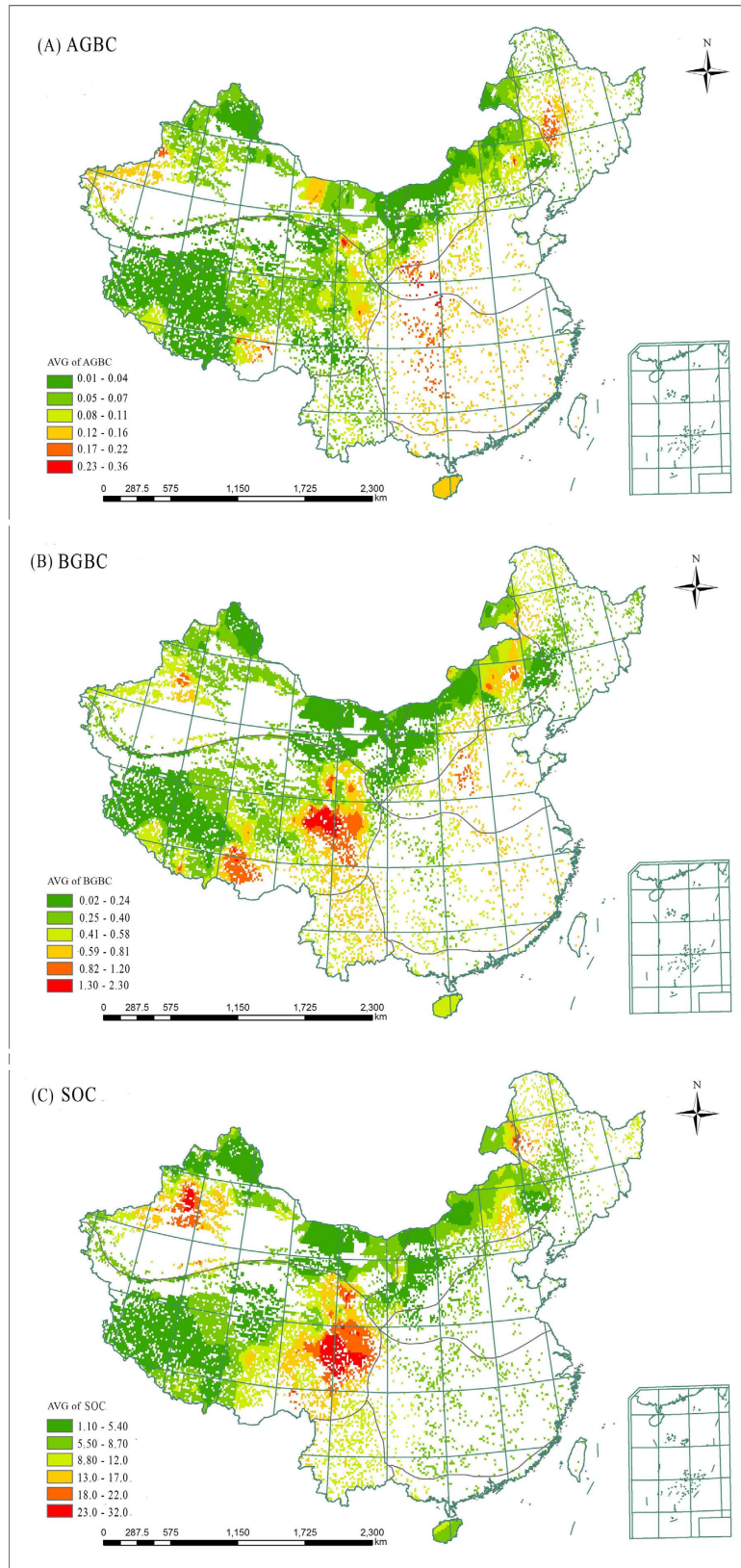


Figure 3. Spatial pattern of carbon density (kg C m^{-2}) in the above-ground biomass (AGBC, **A**), below-ground biomass (BGBC, **B**), and soil (SOC, **C**). The figure was created using Arcgis 8.2 software (ESRI Inc., Redlands, CA).

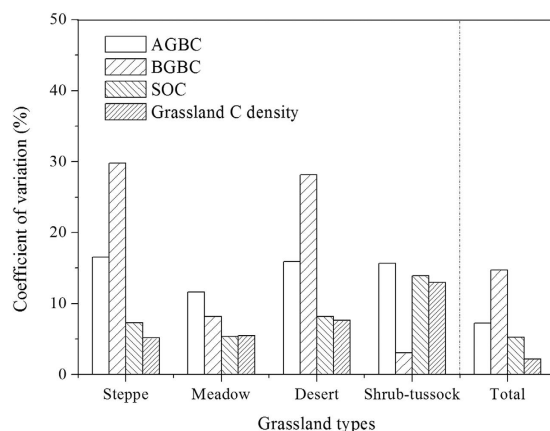


Figure 4. Coefficient of variation (CV, %) of carbon density on three grassland classification scales in different components and grassland types. AGBC, carbon density of the above-ground biomass; BGBC, carbon density of the below-ground biomass; SOC, SOC density.

| Grassland type groups | Area (10 ⁶ ha) | C density (kg C m ⁻²) | | | | C storage (Pg C) | | | | CV (%) |
|-----------------------|---------------------------|-----------------------------------|--------------------|---------------------|---------------------|------------------|---------|---------|-----------|--------|
| | | AGBC [‡] | BGBC | SOC | Ecosystem | AGBC | BGBC | SOC | Ecosystem | |
| Steppe | 138.20 | 0.059 ^{‡†} | 0.354 [‡] | 6.497 ^{‡c} | 6.910 ^{‡c} | 0.081 | 0.489 | 8.979 | 9.549 | 5.2 |
| | | (0.010) [†] | (0.109) | (0.474) | (0.356) | (0.013) | (0.151) | (0.655) | (0.492) | |
| Meadow | 119.19 | 0.067 ^{‡a} | 0.508 ^b | 11.633 ^b | 12.208 ^b | 0.080 | 0.605 | 13.866 | 14.551 | 5.5 |
| | | (0.008) | (0.041) | (0.622) | (0.668) | (0.009) | (0.049) | (0.741) | (0.797) | |
| Desert | 61.61 | 0.048 ^{‡a} | 0.075 ^c | 5.617 ^a | 5.740 ^a | 0.029 | 0.047 | 3.460 | 3.536 | 7.6 |
| | | (0.008) | (0.021) | (0.460) | (0.438) | (0.005) | (0.013) | (0.283) | (0.270) | |
| Shrub-tussock | 34.77 | 0.139 ^b | 0.718 ^d | 7.292 ^c | 8.148 ^c | 0.048 | 0.250 | 2.535 | 2.833 | 13.0 |
| | | (0.022) | (0.022) | (1.013) | (1.057) | (0.008) | (0.008) | (0.352) | (0.367) | |
| Total | 353.77 | 0.078 | 0.414 | 7.760 | 8.251 | 0.239 | 1.390 | 28.841 | 30.470 | 2.2 |
| | | (0.006) | (0.041) | (0.157) | (0.182) | (0.018) | (0.204) | (0.135) | (0.283) | |

Table 2. Estimation of carbon density and storage of Chinese grasslands using three different grassland classifications (different scales). [†]Data represent the mean and standard deviation (parentheses); data with the same lower letter in the same column indicate no significant differences at the P = 0.05 level. [‡]AGBC, carbon density of the above-ground biomass; BGBC, carbon density of the below-ground biomass.

When combining all grassland areas, the C storage of grassland (including AGB, BGB, and SOC down to the 100 cm soil layer), estimated by the six integrative methods, ranged from 29.49 to 32.53 Pg C, with a mean of 30.98 Pg C (Fig. 6). The C storage in Chinese grasslands was composed of 0.7% AGBC (0.23 ± 0.01 Pg C), 4.5% BGBC (1.38 ± 0.14 Pg C), and 94.8% SOC (29.37 ± 1.20 Pg C). In general, the estimates obtained from the three spatial interpolation methods tended to be higher than those from grassland classification methods. The estimate of M5, which classified Chinese grasslands into 18 types, was closest to the average of grassland C storage calculated by the six methods (30.79 Pg C).

Discussion

This study provided the first assessment of the C density and C storage of all main components in Chinese grasslands at the national scale using the six integrative methods. The results found that uncertainty of C storage estimation in Chinese grasslands, resulting from the selected scale-up methods, was approximately 4.8%. However, the level of uncertainty appeared to vary among the different components of grasslands, with the highest and lowest uncertainty being obtained from BGBC and SOC, respectively. One reason for the high uncertainty of BGBC may be the data distribution of BGBC, as the values varied from 0.002 kg C m⁻² to 3.44 kg C m⁻². Although expanding the soil depth to 100 cm may result in overestimation of BGBC to some extent, the influence on the ecosystem C estimates was not noticeable because of the small contribution of BGBC in deeper soil layers to total BGBC (Supplementary Fig. S1) and the small proportion of vegetation C density in grasslands.

SOC accounted for 94.8% of grassland C in this study, which was similar to those of previous studies that found that soil stores 90–97% of grassland C^{4,18,19}. Considering the importance of SOC for grassland C estimates, regional variation (CV) of the C density of grasslands mainly depended on SOC variation. Up to date, it is impossible to accurately estimate soil C storage in Chinese grasslands by remote-sensing methods (or satellite-based approach). In order to keep the consistency of methods to estimate the C densities of AGBC, BGBC, and SOC, the methods of spatial-interpolation and mean density therefore were used to estimate vegetation C storage in

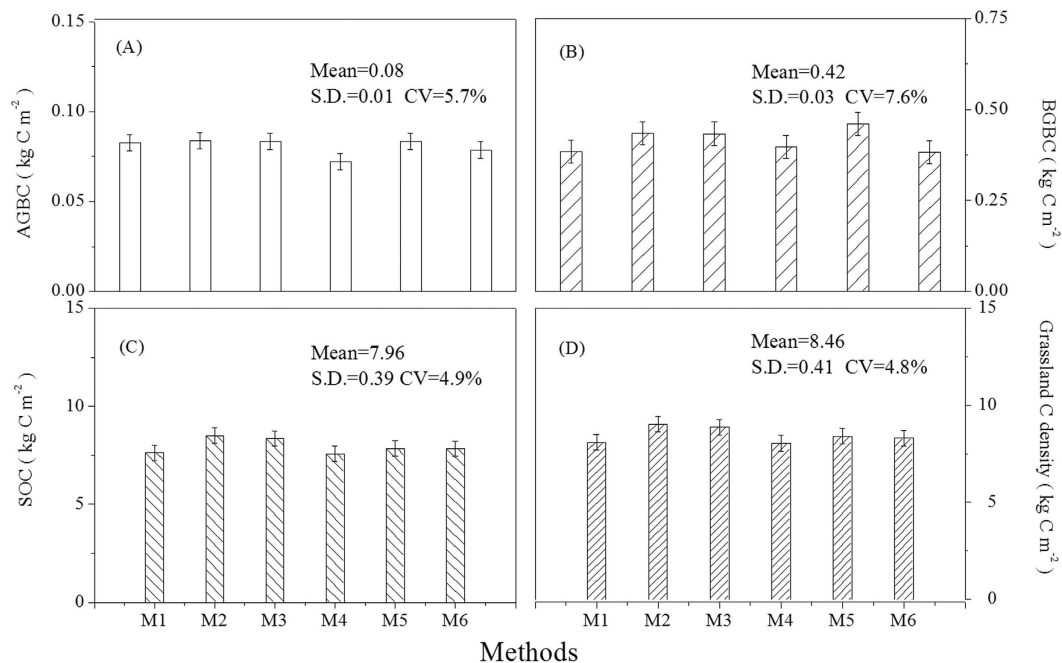


Figure 5. Comparison of carbon density estimated from the six integrative methods. (A) carbon density of the above-ground biomass (AGBC); (B) carbon density of the below-ground biomass (BGBC); (C) SOC density (SOC); (D) grassland carbon density. See Table 4 for methodological descriptions.

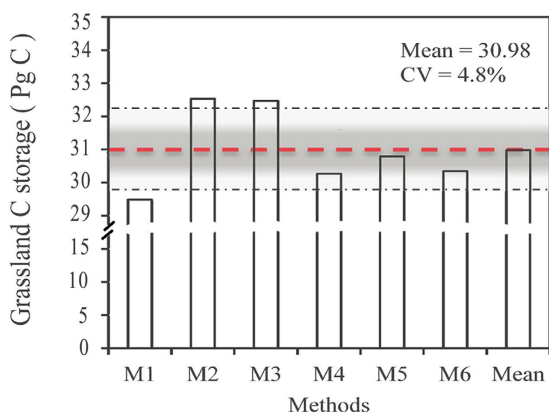


Figure 6. Changes in the estimation of carbon storage in Chinese grasslands for six integrative methods. The methods are described in Table 4. Red line indicates the mean value of the six methods, and the rectangular area is the 95% confidence interval.

this study, although the linkage between field investigation and remote-sensing should improve the estimate of AGBC, to some extent.

The CV values of grassland C density calculated from the three spatial interpolation methods (5.5%) were higher than those calculated from the three grassland classification methods (2.2%). Our results demonstrated that the spatial interpolation methods have higher uncertainty of estimates, because they were more easily affected by the data quantity and spatial distribution of sampling sites²⁰. Furthermore, the spatial interpolation methods generally produced higher estimates (8.66 ± 0.50 kg C m⁻²) of grassland C density than the grassland classification methods (8.25 ± 0.18 kg C m⁻²). One possible explanation is that the spatial interpolation methods may be affected more by the number of sampled sites and nearby sites, particularly those with higher C density. The grassland C estimate was mainly decided by SOC. The SOC estimate in the 0–100 cm soil layer obtained using the six integrative methods was 7.96 kg C m⁻² in this study, which was similar to the previous results of (7.80 kg C m⁻²) Yang *et al.*²³ and (8.50 kg C m⁻²) Fang *et al.*¹⁸. The SOC estimate here was 3.33 kg C m⁻² in the 0–20 cm soil layer, which was about 41.8% of that in the 0–100 cm and was similar to the average value of global grasslands (42%)²⁴. For all methods, the estimation of grassland C storage using 18 vegetation types produced the value closest to the mean of the six methods, and this method was used by Fan *et al.*²¹ and Ni²². This result

| No. | Data and methods | Period | Area (10 ⁶ ha) | Vegetation C density (kg C m ⁻²) | | | Vegetation C storage (Pg C) | | | SOC density (kg C m ⁻²) | SOC storage (Pg C) | Ecosystem storage (Pg C C) | Reference |
|-----|---|--------------------|---------------------------|--|------|------------|-----------------------------|------|------------|-------------------------------------|--------------------|----------------------------|------------|
| | | | | AGBC [‡] | BGBC | Vegetation | AGBC | BGBC | Vegetation | | | | |
| 1 | Global mean C density | 1980s | 405.9 | | | 1.15 | | | 4.66 | | 53.72 | 58.38 | 2 |
| 2 | Global mean C density | 1980s | 299.0 | | | 1.15 | | | 3.06 | 13.10 | 41.03 | 44.09 | 22 |
| 3 | Grassland resource survey | 1980s | 299.0 | 0.06 | | | 0.13 | | | | | | 12 |
| 4 | Grassland resource survey | 1980s | 331.4 | 0.05 | | 0.35 | 0.15 | | 1.15 | | | | 10 |
| 5 | Grassland resource survey & NDVI | 1980s | 331.4 | 0.04 | 0.27 | 0.32 | 0.15 | 0.90 | 1.05 | | | | 13 |
| 6 | Grassland resource survey & NDVI | 1980s | 334.1 | 0.04 | 0.27 | 0.32 | 0.15 | 0.91 | 1.05 | | | | 14 |
| 7 | CEVSA & NDVI | 1981–1998 | 167.0 | | | 0.34 | | | 0.56 | 9.99 | 16.70 | 17.26 | 15 |
| 8 | Grassland resource survey & Field-measured data | 1980s 2003–2004 | 331.0 | 0.12 | 0.88 | 1.00 | 0.39 | 2.92 | 3.32 | | | | 21 |
| 9 | Second soil survey | 1980s | 249.3 | | | | | | | 13.54 | 37.70 | | 26 |
| 10 | Integrating data | 1980s–2000s | 331.4 | | | 0.82 | | | 2.72 | 12.99 | 43.00 | 45.51 | 35 |
| 11 | Field-measured data | 2003–2014 | 355.05 | 0.08 | 0.42 | 0.50 | 0.23 | 1.38 | 1.61 | 7.96 | 29.37 | 30.98 | This study |

Table 3. Estimation of carbon density and storage from different studies of Chinese grasslands[†]. [†]The soil depths for the estimation of SOC density and storage are all approximately 100 cm in this table [‡]AGBC, carbon density of the above-ground biomass; BGBC, carbon density of the below-ground biomass.

indicates that integrative methods require a suitable scale to ensure that sampling sites in a given region are sufficient, particularly when the field-measured sites are uncertain. These findings provide new insights showing that multiple approaches, especially the multiple-scale integrative method, should be used to estimate the C storage in terrestrial ecosystem at large scales.

The estimation of grassland C storage at the national scale was limited and was apparently different among the previous studies (Table 3). One possible explanation is the different data sources for C density¹⁰. Previous studies mainly used data from the Grassland Resource Survey in China to estimate the C density of AGB^{10,13,14,25} (Table 3), and BGB was deduced from AGB using R/S ratios^{13,14}, whereas others used the global mean vegetation C density to estimate vegetation C density^{2,22}. The global mean soil C density^{2,22} or data from the Second Soil Survey in China²⁶ were used to estimate SOC density in Chinese grasslands. Furthermore, Yang *et al.*^{16,17} used field-measured data to estimate the C density of vegetation and SOC in northern China's grasslands, and found that the C density of AGB, BGB, and SOC was approximately 0.04, 0.22, and 8.49 kg C m⁻², respectively. In addition, Yang *et al.*²⁷ estimated Chinese grassland vegetation C storage and SOC storage to be 0.90 Pg C and 35.06 Pg C, respectively, by remote sensing data. Different data sources resulted in different estimates. Therefore, we concluded that these major differences in data sources are important reasons for the high uncertainty of C storage in Chinese grasslands.

A second possible explanation for these differences is the lack of data for key components in grasslands, especially synchronous measurements. Vegetation C density could be estimated by many methods, such as remote sensing, modelling, and field-investigation. However, SOC is difficult to be estimated by remote sensing and modelling, although SOC is the most important component in grassland ecosystems. As shown in Table 3, the calculation of C density in AGB was 0.06 ± 0.03 kg C m⁻² with 47.4% variation, whereas the C density in BGB was 0.41 ± 0.27 kg C m⁻² with 66.4% variation. It was estimated that the C density of BGB was approximately 7 times higher than that of AGB, which was consistent with previous results suggesting that 86–88% of vegetation C density is stored in BGB^{21,28}. However, there are few reports of *in situ* measurements of BGB, despite such measurement being important for the estimation of vegetation C density. The R/S ratio has been verified as a good parameter for inferring BGB at the regional scale; however, this measure also generates uncertainty due to various disturbances (e.g., grazing, mowing). Furthermore, previous studies using global mean soil C density^{2,22} overestimated the C storage in Chinese grasslands (Table 3). Therefore, the high variation in C storage estimates derived from the different components of grasslands among different researches mainly resulted from different or nonsynchronous measurements.

Additionally, this study provided the first estimate of three components by field-measurement data and demonstrated that integrative methods have an important influence on the estimation of grassland C storage at the national scale. However, previous studies only used one method to estimate one or two components. Ni¹² used the Grassland Resource Survey to estimate vegetation C density based on grassland classification integrative methods. In comparison, Piao *et al.*¹⁴ used the same data source to estimate C density based on modeling. Their estimates of AGB in Chinese grasslands were 0.06 kg C m⁻² and 0.04 kg C m⁻². Therefore, multiple approaches should be used to estimate the C storage in terrestrial ecosystems and improve the accuracy of C storage estimates at large scales. The uncertainty of C storage in Chinese grasslands using the six integrative methods was approximately 4.8%. The level of uncertainty differed among different components, with the highest values observed in BGBC. Our findings emphasize the underlying influence of integrative methods for estimating C storage in terrestrial ecosystems at a large scale. Based on the six integrative methods, C storage in Chinese grasslands was estimated as 0.23 ± 0.01 , 1.38 ± 0.14 , 29.37 ± 1.2 , and 30.98 ± 1.25 Pg C in AGBC, BGBC, SOC, and grasslands, respectively. This first assessment of C storage in all main components of Chinese grasslands at the national scale will help to determine the potential roles of Chinese grasslands in response to global climate change.

| Approaches | No. method | Sub-No. | Selected methods | Assumption |
|--------------------------|------------|---------|--|--|
| Spatial interpolation | M1 | 1 | Kriging interpolation | Grasslands vary gradually with climate and have a continuous distribution in China. Theoretically, biomass and C storage from one sampling site may be the most similar to these of the nearest site because of more similar hydrothermal conditions. On the basis of this assumption, the methods of spatial interpolation can be used to estimate the C storage of grasslands in China. |
| | M2 | 2 | Inverse Distance Weighted interpolation | |
| | M3 | 3 | Empirical Bayesian Kriging interpolation | |
| Grassland classification | M4 | 1 | 5 grassland types | Grasslands show different characteristics resulting from climate (temperature and precipitation), topography, and soil. Grasslands therefore can be artificially divided into different types to depict the collective characteristics. On the basis of this assumption, C storage in Chinese grasslands can be calculated based on grassland type and corresponding area at different scales. |
| | M5 | 2 | 18 grassland types | |
| | M6 | 3 | 32 grassland subtypes | |

Table 4. Six integrative methods to estimate C storage in Chinese grasslands.

Methods

Data sources. C density data for different components of Chinese grasslands were derived from: (1) field-measured data of 210 papers publicly published from 2004 to 2014 in the Web of Science (www.Webofknowledge.com) and in the China National Knowledge Infrastructure (<http://epub.cnki.net>) (Supplementary Appx. S1 ~ S4), in addition to (2) some unpublished field-measured data obtained by personal correspondence (Supplementary Appx. S1).

Furthermore, we obtained the Chinese grassland areas and the spatial distribution of different grassland types from the map of grassland resources in China at 1:4M²⁹. Based on this map, the total area of Chinese grasslands was estimated as 3.55×10^8 ha.

Data processing. Field-measured above-ground biomass (AGB, kg m⁻²) records were used directly. If the AGB data were measured monthly in the published papers, we chose the values measured in August, which is commonly considered to be the peak period for AGB in Chinese grasslands⁴. The C content of AGB and below-ground biomass (BGB, kg m⁻²) in grasslands was estimated to comprise 45% of the plant dry matter³; thus, we calculated the C density of AGB (AGBC, kg C m⁻²) from AGB and the coefficient of C content (0.45).

Similarly, the C density of BGB (BGBC, kg C m⁻²) was calculated from BGB and the coefficient of C content (0.45). In practice, the BGB data were directly used if the sampling depth was 100 cm. For data obtained at a soil depth of less than 100 cm, we estimated the BGBC as a 0–100 cm soil layer based on the 0–20 cm data using the following procedure. We first established the relationships of BGBC between 0–20 cm and 0–100 cm using 99 sampling sites where data were simultaneously collected at 20 cm and 100 cm depths. We found that BGBC showed a significantly positive correlation between 0–20 cm and 0–100 cm ($R^2 = 0.97$, $P < 0.001$; Supplementary Fig. S1). Therefore, the BGBC values in the 0–100 cm soil layer may be deduced from surface soil (0–20 cm) data by the following formula:

$$y = 1.095x + 0.08 \quad (1)$$

where y and x are BGBC at the 100 cm and 20 cm depths, respectively.

SOC density (SOC, kg C m⁻²) was calculated using equation 2, unless SOC data were reported directly in the published papers. In practice, we commonly used the field-measured bulk density. For soil samples without bulk density records, we used the mean values of bulk density from the second soil survey in China (1.3 g cm^{-3}) as a substitute³⁰. For soil samples from a depth of less than 100 cm, we adopted the empirical relationships between soil C content and depth to fit to the 100 cm soil layer. In a previous paper, we established the empirical relationships in the 74 terrestrial ecosystems of China using the long-term monitoring data of the Chinese Ecosystem Research Network³¹. Here, we randomly selected 118 sites to validate the accuracy of the prediction, and found that the predicted SOC values are closely correlated and almost equal to the measured values in the 0–100 cm soil layer ($y = 1.08x - 0.19$, $R^2 = 0.95$) (Supplementary Fig. S2).

$$SOC = \sum_{j=1}^n (H_j \times c_j \times b_j \times 0.1) \quad (2)$$

where SOC is SOC density (kg C m^{-2}), H_j is soil thickness (cm), c_j is the concentration of SOC (%), b_j is bulk density (g cm^{-3}).

Overall, the total number of sampling sites for AGBC, BGBC, and SOC was 2659, 1022, and 991, respectively. The distribution of the sampling sites is shown in Fig. 1.

Integrative methods. To assess the influence of different integrative methods on C storage estimates in the scaling-up process in this study, we adopted three spatial interpolation methods and three grassland classification methods (Table 4).

Spatial interpolation methods. The geo-statistical principle assumes that grasslands gradually change with climate. Theoretically, the biomass at one sampling site may have the highest similarity to that at the nearest site. Based on this assumption, spatial interpolation methods may be used to estimate the C storage of grasslands in China. In practice, we selected three spatial interpolation methods, *viz.* Kriging interpolation (M1), Inverse Distance Weighted interpolation (M2), and Empirical Bayesian Kriging interpolation (M3). Among these methods, M2 only considers the distance, whereas M1 and M3 consider both the spatial orientation and the distance.

For statistical and comparative analyses, the grasslands were divided into six regions according to the nationwide grassland resource survey of DAHV & GSAHV²⁵, which were designated as temperate semi-humid meadow steppe regions (R1), temperate semi-arid steppe and desert steppe regions (R2), temperate and warm temperate arid desert and mountain steppe regions (R3), Qinghai-Tibet alpine regions (R4), warm temperate semi-humid and semi-arid shrub-tussock regions (R5), and subtropical and tropical shrub-tussock regions (R6) (Fig. 1).

Grassland classification methods. Grasslands exhibit different ecological characteristics based on climate (temperature and precipitation), topography, and soil. Therefore, grasslands may be divided into different artificial types. Based on this assumption, C storage in Chinese grasslands may be calculated from grassland type and the corresponding area at different scales (Table 4 and Supplementary Appx. S5). The major aim is to explore C storage at the national scale based on the C density of different grassland types.

For the statistical and comparative analyses, the Chinese grasslands were classified at three different scales. First, 5 grassland types of China's vegetation were determined at 1:1000000 resolution³² (M4): steppe, meadow, desert, shrub-tussock, and swamp. Swamps were excluded in this study because of their small area and insufficient sampling sites. Second, 18 grassland types based on the national grassland survey were used²⁵ (M5) (Supplementary Appx. S5). Third, 32 grassland subtypes based on the national grassland survey were used²⁵ (M6).

Statistical analysis. The coefficient of variation (CV, %) was used to assess the uncertainty of the six methods for estimating C storage^{33,34}, which was defined as the ratio of the standard deviation to the mean. The range of AGBC, BGBC and SOC came from the maximal and the minimum value, and the median value calculated by different methods was used. Grassland C storage was summarized from the C storages in AGB, BGB, and SOC in the 0–100 cm soil layer. One-way variance analysis (ANOVA) was used to test for differences in C density in different regions or grassland types. Spatial interpolation and statistical analysis were performed by Arcgis 8.2 (ESRI Inc., Redlands, CA) and SPSS 13 software. Significant differences were defined as $P = 0.05$.

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Author Contributions

A.N.M. and N.P.H. analyzed data and wrote the manuscript text, D.W. created the Figures 1 and 3, S.L.P. provided support on statistic method, and G.R.Y, N.P.H. and A.N.M. designed the study. All authors reviewed the manuscript.

Additional Information

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