

Above- and belowground biomass allocation and its regulation by planting density in six common grass species in temperate China

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Abstract

1. Above- and belowground biomass allocation is an essential plant functional trait that reflects plant survival strategies and affects belowground biomass estimation in grasslands. However, due to the difficulty in distinguishing fine and living roots, field-based studies show large uncertainties in estimating the biomass allocation. In addition, how plant density regulates biomass allocation across various species remains poorly addressed. 2. To address this knowledge gap, we conducted greenhouse manipulation experiments to explore patterns of above- and belowground biomass allocation and its regulation by planting density in six common grass species of different functional types (i.e., C₃ vs C₄; annuals vs perennials) in China's temperate grasslands. The six species were *Chenopodium glaucum*, *Cleistogenes squarrosa*, *Leymus chinensis*, *Medicago sativa*, *Setaria viridis*, and *Stipa grandis*. 3. We found that the mean values of root to shoot ratio (R/S) ranged from 0.04 to 0.92 across the six species, with much lower R/S values in annuals than in perennials (*C. glaucum* and *S. viridis* vs *C. squarrosa*, *L. chinensis*, *M. sativa* and *S. grandis*) and in C₄ plants than in C₃ plants (*C. squarrosa* vs *L. chinensis*, *M. sativa* and *S. grandis*). For *C. squarrosa*, increasing the planting density decreased the shoot biomass fraction (SMF) but increased the root biomass fraction (RMF) and R/S. In contrast, for the other five species, planting density had nonsignificant effects on the SMF, RMF, and R/S. In addition, the planting density significantly affected the allometric relationships between above- and belowground biomass. 4. Synthesis. Our results suggest that R/S values obtained from field investigations are severely overestimated and that the pattern and density regulation of the biomass allocation vary across species of different functional types. Our findings provide important insights into approximating difficult-to-measure belowground biomass with easier-to-measure aboveground biomass in grassland ecosystems, and also provide important theoretical foundations for grass-based livestock husbandry practices.

1. Introduction

The allocation of biomass to above- and belowground organs is one of the most important functional traits in plants (Dolezal et al., 2020; Enquist & Niklas, 2002). This trait not only reflects plant survival strategies but also has a significant influence on soil carbon (C) inputs (Poeplau, 2016; Rasse et al., 2005; Umaña et al., 2020). Grasslands are the most widespread terrestrial ecosystem and have important regulatory functions in the global C cycle (Dixon et al., 2014; White et al., 2000). The accurate assessment of the size of C pools in belowground biomass is an important issue for future research on grassland C cycling (Ahlstrom et al., 2015; Mokany et al., 2006). Due to the difficulty of root sampling, the belowground biomass of grasslands is usually indirectly estimated with the aid of the easier-to-measure aboveground biomass and empirical root to shoot ratio (R/S), which has become a key parameter for estimating the size of grassland C pools (Jackson et al., 1996; Mokany et al., 2006). Therefore, exploring the above- and belowground biomass allocation patterns of

herbaceous plants and their modulating factors is pivotal for deepening our understanding of plant growth, survival, and carbon cycling in terrestrial ecosystems (Poeplau, 2016).

For grassland ecosystems, the R/S reported from previous field surveys is generally greater than 1 and varies widely among different studies. For example, Jackson et al. (1996) reported that the global mean value of R/S was 3.7; Mokany et al. (2006) combined data from community investigations and calculated a global mean R/S of 4.5 for temperate grassland communities. Yang et al. (2010) estimated a mean R/S value of 6.3 for grassland communities in China using field investigations from 265 sample sites, while Ma et al. (2006) obtained a mean R/S value of 61.3 for the Inner Mongolian grassland based on the synthesis of field-measured data; the R/S value at some of these sample sites reached over 400. In addition, results at the genus level differ greatly from those at the community level. For example, a study based on grasslands in northern China showed that the R/S of the four dominant genera (*Stipa spp.*, *Cleistogenes spp.*, *Agropyron spp.*, and *Leymus spp.*) ranged from 0.75 to 2.98 (Luo et al., 2013), while the median R/S at the individual level for this region was only 0.84 (Wang et al., 2010). Although a large amount of observational data has been accumulated globally over the past few decades, it is still difficult to reach a unified understanding at the global scale through data syntheses, because large unreliable datasets can deteriorate accurate statistical analyses and interpretations of the results (Mokany et al., 2006). In addition, community-level studies are restricted to reporting the weighted mean value of R/S for a given community without considering the large variations in R/S at the intra- and interspecific levels. To date, the reasons for the discrepancies in these results among different studies are unclear, which presumably results in large uncertainties in estimating the belowground C pools in grasslands. Therefore, accurate manipulation experiments are necessary to broaden our understanding of above- and belowground biomass allocation across different species.

Previous studies have overwhelmingly concluded that plant biomass allocation is jointly determined by both environmental and biotic factors (Agathokleous et al., 2019; Gedroc et al., 1996; Poorter et al., 2012). In terms of environmental factors, numerous studies have shown that water conditions (Eziz et al., 2017), temperature (Reich et al., 2014; Wang et al., 2016), nitrogen and phosphorus availability (Müller et al., 2000; Peng & Yang, 2016; Yan et al., 2019) and their ratios (Kumar et al., 2020), CO₂ concentrations (Pei et al., 2020), and soil texture (Xie et al., 2012) can have significant effects on R/S. Among those biotic factors, planting density is not only an important selection pressure in nature but also an important tool for artificial pasture management (Boschma et al., 2019). It has been well recognized that planting density can alter the above- and belowground competition for light and nutrient among plants (Kira et al., 1953; Weiner, 1986; Weiner & Freckleton, 2010; Yoda et al., 1963) and thus influences biomass allocation. Through a global data synthesis analysis, Poorter et al. (2012) found that on average, plants tended to increase their R/S with increasing planting density. However, many site- or species-specific studies have also found very complicated density effects on R/S, with invariant (Casper et al., 1998), increasing (Berendse & Möller, 2009) and decreasing (Hecht et al., 2016; Weiner, 1990) trends with increasing planting density. However, the density effects on herbaceous plant biomass allocation, particularly across multiple species of different plant functional types, are still less well understood.

To address the knowledge gap mentioned above, we conducted a greenhouse experiment with a total of six common species planted in five density gradients. These species represent different plant functional types in the temperate grasslands in northern China. We cultivated these plants to the maturity stage and harvested the shoot and root biomass from individuals of each species. We aim to address the following two questions: (1) How does above- and belowground biomass allocation vary among different plant functional types? and (2) How does planting density regulate above- and belowground biomass allocation in these species?

2. Materials and Methods

2.1 Study species and experimental design

To increase the generality of our results, we selected six common grass species with different functional traits (different photosynthetic pathways (C₃ vs C₄), life forms (annual vs perennial), and root system characteristics (rhizome vs bunch)) that grow in temperate grasslands in China. These species are *Chenopodium*

glaucum , *Cleistogenes squarrosa* , *Leymus chinensis* , *Medicago sativa* , *Setaria viridis* , and *Stipa grandis* . Among these species, *M. sativa* is a high-quality forage species and is widely grown in artificial grasslands, while the other five species are common in natural grasslands (Figure 1).

The pot experiment was conducted in the greenhouse of the Institute of Botany, Chinese Academy of Sciences, Beijing, China (39°9' N, 116deg49' E). A homogenous substrate was prepared by mixing sand, vermiculite (3-5 mm diameter to prevent the roots from sticking to the substrate), and peat soil (Jiffy, Netherlands) at a ratio of 3:1:1 by volume. The planting pots (length: 40 cm x width: 40 cm x height: 30 cm; volume: 48 L) were filled with ~16 kg of the mixed substrate. In this experiment, five densities were established for each species: 25 (D1), 100 (D2), 225 (D3), 400 (D4), and 625 (D5) plants m⁻². There were three replicates for each density level and a total of 90 pots for all the species and density levels. The number of plants sown per pot was determined by the planting density and the pot area (0.16 m²); in total, there were 3,960 individuals. The experiment was conducted using a randomized block design with one block per species for a total of six plots (Figure 2).

2.2 Cultivation and harvest

To avoid root damage from transplanting and to improve seedling survival, we established monocultures by sowing the seeds and then thinning the seedlings. Before sowing, the surface of the planting pots was evenly gridded using yarn according to the number of seeds to be sown. All the seeds were sterilized with 75% alcohol solution for 10 minutes and then washed with distilled water three times. Then, the seeds were placed into the corresponding grid to a depth of approximately 1 cm from the surface of the substrate. A backup pot was prepared for each species, and seedlings were replanted if they died within two weeks after thinning (Figure S1). The average day/night temperature in the greenhouse was 28.5/18.5 degC, and the relative humidity was between 40% and 60%. The positions of the pots were randomly changed within and between blocks every two weeks. Each pot was watered with a measured amount every 3 to 5 days to ensure plant growth. In the middle and late cultivation stages, we raised the planting pots with nylon netting to prevent 'competition escape' of aboveground organs and to make the conditions as similar to field conditions as possible (Figure S2).

The experiment was conducted from August 19, 2019, to January 12, 2020. As the annuals (*C. glaucum* and *S. viridis*) had relatively short life spans, they were harvested in the eighth week. Before harvesting, each plant in the planting pots was numbered according to its grid position and labelled. The reproductive organs were harvested first, followed by the leaves, stems and roots. These separate organs were placed into corresponding envelope bags. The root samples were individually placed in a 2-mm nylon mesh sieve and washed with tap water. Afterwards, all the samples were dried at 65 degC (for 72 h) until reaching a constant mass. The aboveground biomass of an individual plant included the seeds, stems, and leaves, and the belowground biomass included only roots for most species; the exception was *L. chinensis* , whose belowground biomass included both rhizomes and roots.

2.3 Vegetation productivity data

The peak biomass during the growing season was considered the net primary productivity (NPP) because all plants in this study were cultivated from seeds (Scurlock et al., 2002; Singh et al., 1975). Comparing the species-level above- and belowground NPP allocation with that from field observations of grass communities at the global scale can help us understand the mechanisms of variation between results at different study scales. It is also possible to further verify whether the pattern of experimental data from greenhouse manipulations can be extended to the field. Two types of sources for paired aboveground NPP (ANPP) and belowground NPP (BNPP) data at the field community level were used in this study: online databases and literature-reviewed data. The online data were obtained primarily from the Oak Ridge National Laboratory Distributed Active Archive Center (ORNLDAAC) (<http://daac.ornl.gov>), which contains three datasets (Esser, 2013; Olson et al., 2013; Scurlock et al., 2015). The literature-reviewed data were obtained primarily from ISI-Web of Science (Thomson Reuters, New York, NY, USA), Google Scholar (Google Inc., Mountain View, CA, USA) and the China National Knowledge Infrastructure (<http://epub.cnki.net>). Details regarding

the extraction processes and initial screening criteria for these data are described in Sun et al. (2020).

2.4 Statistical analyses

We performed two main analyses in this study. First, one-way ANOVA combined with a multiple comparisons test (least significant differences, LSD) was used to test the differences in the fraction of total plant biomass allocated to shoots (the shoot mass fraction, SMF), the fraction of total plant biomass allocated to roots (the root biomass fraction, RMF), and the R/S among the different species and densities. Second, standard major axis (SMA) regression analyses were conducted to explore the allometric relationships of biomass between shoots and roots at different densities. A likelihood ratio test was used to indicate the heterogeneity of the scaling exponents at different densities and their difference from 1 (Warton et al., 2012). The data were \log_{10} -transformed before the SMA regression analysis. The differences in the SMF, RMF and R/S among the six species were analysed based on the pooled data from all five planting densities. The pooled data were subjected to a Kolmogorov-Smirnov test to examine whether they conformed to a normal distribution; if they did not, a \log_{10} transformation was performed. Before using ANOVA to study the effect of density on the biomass allocation in each species, the data from the individuals in each planting pot were averaged; therefore, the data presented in the figures are averages based on three replicates. In addition, differences in the ANPP and BNPP fractions and ratios for the different functional groups (annuals *vs* perennials, C_3 *vs* C_4) at the global scale were analysed using a mixed model and compared with the results of this experiment. All analyses in this study were performed in R 4.0.2 (R Core Team, 2020).

3. Results

3.1 Above- and belowground biomass allocation

There were significant differences in the SMF, RMF and R/S among the six species ($P < 0.001$, Figure 3). The average RMF was the highest in *M. sativa* (46.5%), followed by *L. chinensis* (38.2%), *S. grandis* (36.3%), *C. squarrosa* (24.0%), *S. viridis* (3.7%) and *C. glaucum* (3.6%). The R/S were ranked in the order *M. sativa* (0.92) > *L. chinensis* (0.72) > *S. grandis* (0.64) > *C. squarrosa* (0.33) > *S. viridis* (0.04) > *C. glaucum* (0.04). The shoot biomass was the dominant fraction in the two annuals (*S. viridis* and *C. glaucum*), and there was a statistically insignificant difference between them ($P > 0.05$). In contrast, the SMFs of perennials (*M. sativa*, *L. chinensis*, *S. grandis* and *C. squarrosa*) were all significantly smaller than those of annuals ($P < 0.001$) but greater than their respective RMFs. In addition, the perennial C_3 plants (*M. sativa*, *L. chinensis* and *S. grandis*) tended to allocate more biomass to roots than the perennial C_4 plant (*C. squarrosa*) (Figure 3).

3.2 Density effects on above- and belowground biomass allocation

The density effects on the above- and belowground biomass allocations varied among the different species. There were statistically insignificant changes in the total biomass of *S. grandis* and the root biomass of *S. viridis* per individual with increasing density (Figure 4c, k), while the average total, shoot and root biomass of the other four species per individual showed decreasing trends with increasing density (Figure 4a, e, g, i). Density had a very limited effect on the biomass fraction of the annuals (*C. glaucum*, *S. viridis*) and *S. grandis*, and the SMF, RMF and R/S remained almost unchanged under the different density conditions (Figure 4b, d, l). In contrast, for the other perennials, density had significant effects on the biomass fractions, but these effects showed different patterns. For *C. squarrosa*, the SMF decreased while the RMF and R/S increased with increasing density (Figure 4f). For *L. chinensis* and *M. sativa*, density had complicated effects on the SMF, RMF and R/S; each of these indicators tended to fluctuate with increasing density (Figure 4h, j).

3.3 Density effects on the allometric relationships between shoot and root biomass

The density effects on the allometric relationships were inconsistent across the six species (Table 1; Figure 5). Among them, the scaling exponents of *S. viridis*, *C. squarrosa* and *L. chinensis* did not differ significantly among the five planting densities (Figure 5b-d). Density had a significant effect on the scaling exponents of

C. glaucum and *S. grandis*, but the scaling exponents fluctuated with increasing density (Figure 5a, f). In *M. sativa*, the scaling exponents gradually decreased with increasing density (Figure 5e).

4. Discussion

4.1 Large discrepancies between the R/S ratios from greenhouse experiments and field investigations

In this study, we found that the individual-level mean R/S values for the six common species from the temperate grasslands in northern China ranged from 0.04 to 0.92 (Figure 3), which were lower than those from global and regional field community investigations (Table 2). Specifically, our result is much lower than that (1.66~15.21) of a field community survey performed in the same region (Wang et al., 2008); the R/S of perennials in this study is close to the result (0.84) of individual-level field observations (Wang et al., 2010) but still lower than that (0.75~2.98) of genus-level surveys (Luo et al., 2013). Considering the field sampling process, we suggest that the R/S obtained in field community studies may be strongly overestimated for three main reasons. First, field sampling methods are usually not equipped to accurately identify dead and live roots and are more prone to including dead roots in root samples, resulting in an overestimation of root biomass and therefore of R/S (Wang et al., 2010). Indeed, the large discrepancy between the R/S results at the individual and community levels in the same area can also support this view. For example, Wang et al. (2010) reported that the median R/S at the individual level in northern China's grasslands was only 0.84, which was much lower than that at the community level (Table 2). In fact, root sampling at the individual level is more accurate than sampling at the community level because root systems of the same species are relatively uniform in morphology and colour, which makes it easier to distinguish living roots from dead roots. Second, grazing by herbivores can reduce shoot biomass to some extent, resulting in an underestimation of shoot biomass and the subsequent overestimation of R/S (Mikola et al., 2009). Third, for mature communities in the field, the dominant species are mainly perennials, and there are much fewer annuals. Our study found that the RMFs of annual grasses were extremely low. Therefore, if the R/S derived from perennial plant samples is used to estimate the root biomass of annuals, it will be highly biased.

The overestimation of R/S can occur during field sampling, but greenhouse pot experiments can effectively circumvent some of the shortcomings of field sampling. First, the roots of greenhouse plants can be fully sampled and estimated. The cultivation substrate in this study was homogeneous and uncomplicated and could be easily separated from the root system. Second, the greenhouse growth environment is consistent and stable among replicates; shoot biomass is not lost due to animal grazing, and the influence of microenvironmental variability on biomass allocation is avoided. We also acknowledge that greenhouse cultivation experiments have some drawbacks, such as the possibility of underestimating root biomass due to the height limitation of the planting pots, which limits the root growth space to some extent. In addition, for perennials, cultivation for only one growing season may result in some underestimation of root biomass if the root turnover time is greater than one year (Gill & Jackson, 2000). However, despite certain drawbacks, greenhouse cultivation experiments may still be a more accurate method than field sampling for measuring the R/S of different species in the future.

4.2 Changes in the above- and belowground biomass allocation fractions among different functional types

The RMFs of the two annuals in this study, *C. glaucum* and *S. viridis*, were less than 4%, which was much lower than their SMFs (Figure 3). Since this experiment lasted for only one growing season, the peak biomass represents the NPP. The patterns of above- and belowground biomass allocation for annuals were further supported by findings at the community level. We synthesized paired ANPP and BNPP data from global grassland communities and found that despite interference from other nondominant species (e.g., perennials), BNPP was much lower than ANPP in grassland communities where annuals were the dominant species (Figure 6a-c); this finding is consistent with the overall trend in annual plant biomass allocation in this study. This pattern may be related to the opportunistic survival strategies of annuals. Due to their rapid growth and short life history cycles, they need to allocate more photosynthetic products

to shoots to maximize photosynthesis and reproductive yield (Grime, 2001). In addition, annuals are less tolerant to environmental stress than perennials, and the belowground parts of annuals often have no energy storage organs; together, these factors result in the aboveground biomass of annuals being larger than their belowground biomass (Gedroc et al., 1996; Zhou et al., 2014). In contrast, perennials in arid and semiarid grasslands are often water- and nutrient-limited during growth processes and generally opt for a ‘conservative’ strategy, allocating a large proportion of their productivity to the root system in order to better adapt to drought conditions or nutrient-poor environments (Balachowski & Volaire, 2018).

The RMF and R/S of the perennial C_4 plant *C. squarrosa* were significantly lower than those of the three other C_3 perennials in this study (Figure 3). This result is consistent with the results from global field community studies showing that the BNPP/ANPP ratio was lower in communities dominated by C_4 plants than in communities dominated by C_3 plants (Figure 6d-f). This may be attributed to the fact that plants have evolved thinner roots throughout their evolutionary history; C_4 plants, which are recognized as being younger than C_3 plants, consistently have thinner absorbing roots and higher water and nitrogen uptake efficiencies than C_3 plants (Edwards et al., 2004; Ma et al., 2018; Osborne, 2008). In addition, the C_4 photosynthetic pathway has a higher affinity for CO_2 than the C_3 photosynthetic pathway, which allows C_4 plants to use smaller stomatal openings for CO_2 uptake and minimize their water losses through transpiration (Chapin et al., 2011). Therefore, C_4 plants do not require excessive C to be allocated to their roots.

4.3 Density effects on above- and belowground biomass allocation and allometric relationships

Our greenhouse manipulation experiment revealed three trends in the RMF and R/S with increasing density among the studied species: statistically insignificant changes (*C. glaucum*, *L. chinensis* and *S. grandis*, Figure 4b, h, l), an increasing trend (*C. squarrosa*, Figure 4f) and fluctuating patterns (*S. viridis* and *M. sativa*, Figure 4d, j). This result suggests that density has different effects on biomass allocation across species. For *C. glaucum*, *L. chinensis* and *S. grandis*, the density-induced above- and belowground competition was symmetrical (Weiner & Thomas, 1986). For *C. squarrosa*, the RMF and R/S increased with increasing density, indicating that competition was more intense belowground than aboveground at higher densities; relatedly, a higher proportion of biomass must be allocated to roots to achieve more efficient nutrient and water uptake (Berendse & Moller, 2009). The relative intensity of above- and belowground competition in *S. viridis* and *M. sativa* varied with density. For example, *M. sativa* had the highest R/S at a moderate density (D3, 225 plant m^{-2}). This density may be the threshold value for above- and belowground biomass allocation in *M. sativa*; when planting densities are close to this value, this species is prone to allocate more biomass to its roots, which is not conducive to increasing forage yields.

Our results further showed that planting density significantly regulated the allometric scaling exponents of shoot biomass against root biomass for *C. glaucum*, *M. sativa* and *S. grandis* (Table 1; Figure 5). These patterns do not support the ontogenetically fixed scaling exponents predicted by allometric partitioning theory, which suggests that scaling relationships are insensitive to biotic or abiotic factors (McCarthy & Enquist, 2007; Muller et al., 2000; Weiner, 2004). Rather, the allometric relationships in this study were altered by the planting density. This inconsistency is likely due to that these three species do not possess the typical fractal structure (e.g., *S. grandis* has an expanded tiller node rather than a fractal structure) that is the basis for the vascular plant allometry model (Wang et al., 2010; West et al., 1999). In contrast, for *S. viridis*, *C. squarrosa* and *L. chinensis*, the planting density had statistically insignificant effects on the allometric scaling exponents of shoot biomass against root biomass; for *M. sativa*, the scaling exponents gradually decreased with increasing density (Table 1; Figure 5). This difference suggests that the effects of planting density on plant allometric relationships between above- and belowground biomass are species-specific. The stability or instability of the scaling exponent response to planting density might be attributed to the balanced or unbalanced limitations on light and nutrients under different competitive conditions (Poorter et al., 2015). Overall, the allometric partitioning theory derived from global data analyses of vascular plants is not applicable to the studied herbaceous plants, and the effects of planting density on allometric relationships should be considered when using predefined scaling relationships to quantify the root biomass of herbaceous plants (Yan et al., 2019).

5. Conclusions

In this study, we conducted experimental manipulations combining five density gradients and six common species of different plant functional types from grasslands in temperate China. Our results showed that the amount of photosynthetic products allocated belowground for each species was lower than that allocated aboveground, particularly for the annuals. Among the different plant functional groups, the R/S of annuals is much lower than that of perennials, suggesting that estimating the belowground biomass of annual-dominant communities with the empirical R/S of perennial plant communities in the field can induce large uncertainties. The R/S of C_4 plants is lower than that of C_3 plants, suggesting that the expansion of C_4 herbaceous plants may have large impacts on future belowground C pool dynamics of grassland ecosystems in the future. The allometric relationships between shoot and root biomass obtained in this study were species-specific and could be modulated by density, which is not in line with allometric partitioning theory. These findings highlight the need to consider the effects of plant functional type and density on above- and belowground biomass allocation patterns when estimating and predicting the future belowground C dynamics of grasslands in the context of climate change.

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Author contributions

J.F. and Y.S. designed the research. Y.S. and Y.W. conducted the greenhouse experiments. Y.S. analysed the data. Y.S., J.F. and Z.Y. wrote the manuscript. All authors contributed substantially to discussion and revision. The authors declare that there is no conflict of interest.

Data accessibility

All data from the manuscript will be uploaded to the Dryad Digital Repository as soon as this manuscript is accepted.

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Table 1 Summary of the standard major axis (SMA) regressions about the allometric relationships between shoot and root biomass (\log_{10} Shoot biomass = $a * \log_{10}$ (Root biomass + β) of the six species based on the pool data (all) and the five density treatments, respectively.

| | Density | a_{SMA} (95% CI) | β_{SMA} (95% CI) | R^2 |
|-------------------------------|---------|---------------------------|-------------------------------|--------------------|
| <i>Chenopodium glaucum</i> | All | 1.028 (0.988, 1.069) | 1.527 (1.435, 1.619) | 0.75*** |
| | D1 | 0.770b (0.587, 1.011) | 1.190c (0.862, 1.519) | 0.85*** |
| | D2 | 1.024a (0.917, 1.144) | 1.484b (1.281, 1.687) | 0.86*** |
| | D3 | 0.851b (0.771, 0.939) | 1.262c (1.080, 1.443) | 0.75*** |
| | D4 | 1.011a (0.919, 1.112) | 1.516a (1.289, 1.743) | 0.59*** |
| | D5 | 0.988a (0.922, 1.058) | 1.380b (1.222, 1.539) | 0.65*** |
| <i>Setaria viridis</i> | All | 1.179 (1.115, 1.247) | 1.927 (1.76, 2.094) | 0.50*** |
| | D1 | 0.912a (0.481, 1.731) | 1.457d (0.097, 2.816) | 0.05 ^{ns} |
| | D2 | 1.288a (1.063, 1.562) | 1.994b (1.440, 2.548) | 0.57*** |
| | D3 | 1.163a (1.014, 1.333) | 2.006a (1.626, 2.386) | 0.50*** |
| | D4 | 1.236a (1.097, 1.392) | 1.988c (1.620, 2.357) | 0.34*** |
| | D5 | 1.142a (1.043, 1.251) | 1.866d (1.589, 2.143) | 0.44*** |
| <i>Cleistogenes squarrosa</i> | All | 0.951 (0.913, 0.991) | 0.462 (0.41, 0.515) | 0.71*** |
| | D1 | 0.598a (0.375, 0.952) | 0.482a (0.282, 0.682) | 0.53** |
| | D2 | 0.802a (0.624, 1.029) | 0.470b (0.282, 0.659) | 0.27*** |
| | D3 | 0.792a (0.697, 0.900) | 0.304d (0.202, 0.406) | 0.56*** |
| | D4 | 0.831a (0.754, 0.916) | 0.268e (0.157, 0.379) | 0.54*** |
| | D5 | 0.857a (0.799, 0.919) | 0.316c (0.226, 0.405) | 0.63*** |
| <i>Leymus chinensis</i> | All | 1.021 (0.975, 1.068) | 0.248 (0.197, 0.299) | 0.66*** |
| | D1 | 0.597a (0.317, 1.123) | 0.447 (0.347, 0.547) | 0.08 ^{ns} |
| | D2 | 1.114a (0.847, 1.467) | 0.251 (0.146, 0.357) | 0.16** |
| | D3 | 1.069a (0.91, 1.255) | 0.342 (0.178, 0.505) | 0.30*** |
| | D4 | 1.046a (0.94, 1.164) | 0.199 (0.091, 0.308) | 0.45*** |
| | D5 | 0.979a (0.892, 1.075) | 0.218 (0.097, 0.338) | 0.34*** |
| <i>Medicago sativa</i> | All | 0.764 (0.738, 0.79) | -0.089 (-0.109, -0.069) | 0.80*** |
| | D1 | 2.152a (1.142, 4.057) | 0.170a (-0.047, 0.386) | 0.08 ^{ns} |
| | D2 | 0.758b (0.663, 0.866) | -0.027b (-0.061, 0.007) | 0.80*** |
| | D3 | 0.858b (0.784, 0.940) | -0.057c (-0.094, -0.020) | 0.78*** |
| | D4 | 0.680b (0.638, 0.725) | -0.170c (-0.204, -0.137) | 0.80*** |
| | D5 | 0.618c (0.580, 0.657) | -0.212c (-0.246, -0.178) | 0.70*** |
| <i>Stipa grandis</i> | All | 1.131 (1.062, 1.205) | 0.371 (0.304, 0.439) | 0.60*** |
| | D1 | 1.139b (0.780, 1.662) | 0.487b (0.117, 0.857) | 0.70*** |
| | D2 | 1.733a (1.417, 2.12) | 0.756a (0.506, 1.006) | 0.58*** |
| | D3 | 1.080b (0.970, 1.202) | 0.273d (0.169, 0.377) | 0.75*** |
| | D4 | 1.180b (1.027, 1.357) | 0.412c (0.271, 0.553) | 0.57*** |
| | D5 | 1.102b (0.981, 1.239) | 0.371c (0.234, 0.508) | 0.45*** |

Note: Different lowercase letters after scaling slopes indicate significant difference ($P < 0.05$) based on a likelihood ratio test.

Table 2 Summary for root shoot biomass ratio (R/S) of grasslands at individual and community scales in different regions.

| Region | Scale | R/S | References |
|--------------------------------|------------|-------------------|----------------------|
| Global | community | 3.7 | Jackson et al., 1996 |
| China | community | 24.6 (2.4~52.3) | Fan et al., 2008 |
| China | community | 7.7 (5.3~10.1) | Piao et al., 2007 |
| China | community | 6.3 (0.4~14.3) | Yang et al., 2010 |
| Northern China | community | 1.66~15.21 | Wang et al., 2008 |
| Inner Mongolia | community | 61.3 (1.4~401.7) | Ma et al., 2006 |
| North America | community | 4.4 | Coupland (1979) |
| Central Europe | community | 3.0 | Coupland (1979) |
| West Europe | community | 4.3 | Coupland (1979) |
| Tropical/subtropical grassland | community | 1.89 (0.38~4.92) | Mokany et al., 2006 |
| Temperate grassland | community | 4.22 (1.59~9.88) | Mokany et al., 2006 |
| Cool temperature grassland | community | 4.50 (0.83~26.03) | Mokany et al., 2006 |
| Savanna | community | 0.64 (0.40~1.08) | Mokany et al., 2006 |
| Northern China | genus | 1.83 (0.75~2.98) | Luo et al., 2013 |
| Northern China | individual | 0.84 | Wang et al., 2010 |
| Northern China (experiment) | individual | 0.04~0.92 | This study |

Figure legends

Figure 1 Characteristics of the six species cultivated in this study.

Figure 2 Schematic diagram of the experimental design. Each species contains five density gradients with three replicates, and a total of 15 plant pots are randomly placed to constitute a plot. D1: the lowest density treatment, with four plants per pot, equal to 25 plants per m^2 as the area of the pot is $0.16 m^2$, and so on.

Figure 3 Frequency distributions of above- and belowground biomass allocation for the six species. (a) Shoot biomass fraction (SMF), (b) root biomass fraction (RMF), and (c) root to shoot ratio (R/S). Numbers in brackets identify the total samples. Different lowercase letters indicate significant differences ($P < 0.05$) among the six species based on one-way ANOVA and the least significant difference post hoc test. R/S was first \log_{10} -transformed to improve normality. For LC, root biomass contains both roots and rhizomes. CG, *Chenopodium glaucum*; SV, *Setaria viridis*; CS, *Cleistogenes squarrosa*; SG, *Stipa grandis*; LC, *Leymus chinensis*; MS, *Medicago sativa*.

Figure 4 Changes in the biomass and biomass fractions with increasing planting density for the six species. Points and error bars denote the means and standard errors, respectively ($n=3$). Different lowercase letters indicate significant differences ($P < 0.05$) among the five gradients of planting density based on one-way ANOVA and the least significant difference post hoc test. For LC, root biomass contains both roots and rhizomes. (a-b) *Chenopodium glaucum* (CG), (c-d) *Setaria viridis* (SV), (e-f) *Cleistogenes squarrosa* (CS), (g-h) *Stipa grandis* (SG), (i-j) *Leymus chinensis* (LC), and (k-l) *Medicago sativa* (MS).

Figure 5 Allometric relationships between shoot and root biomass for the six species based on the data subset from each density treatment. Standard major axis (SMA) regression is used to derive the fitted line. The dashed line represents a 1:1 line. For LC, root biomass contains both roots and rhizomes. (a) *Chenopodium glaucum* (CG), (b) *Setaria viridis* (SV), (c) *Cleistogenes squarrosa* (CS), (d) *Stipa grandis* (SG), (e) *Leymus chinensis* (LC), and (f) *Medicago sativa* (MS).

Figure 6 Frequency distributions of the ANPP, and BNPP fraction, and BNPP/ANPP ratio between different functional groups using online databases and literature-reviewed data. (a) ANPP fraction for annual *vs* perennial; (b) BNPP fraction for annual *vs* perennial; (c) BNPP/ANPP ratio for annual *vs* perennial; (d) ANPP fraction for C₃ *vs* C₄; (e) BNPP fraction for C₃ *vs* C₄; (f) BNPP/ANPP ratio for C₃ *vs* C₄. Numbers identify the total samples. The statistical test between the two groups is based on a linear mixed effects model (generated using the ‘lmer’ function in R) with sites as the random effect. BNPP/TNPP ratios were first log₁₀-transformed to improve normality. ***, $P < 0.001$.

Figure 1

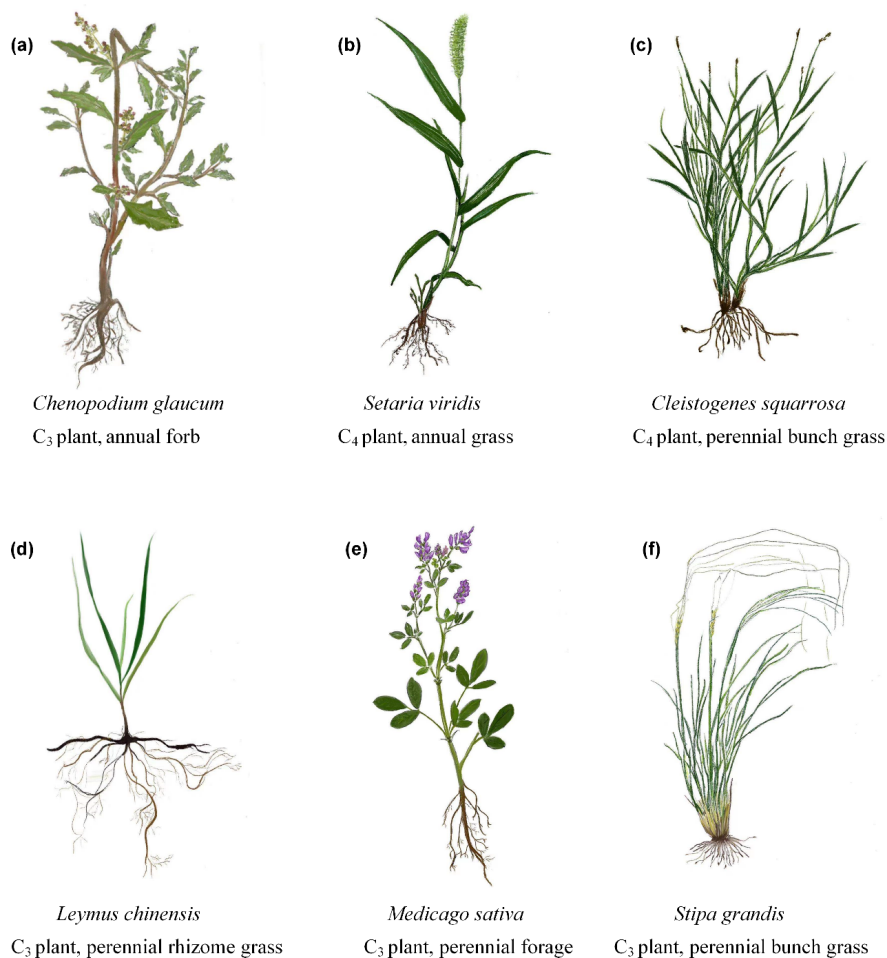


Figure 2

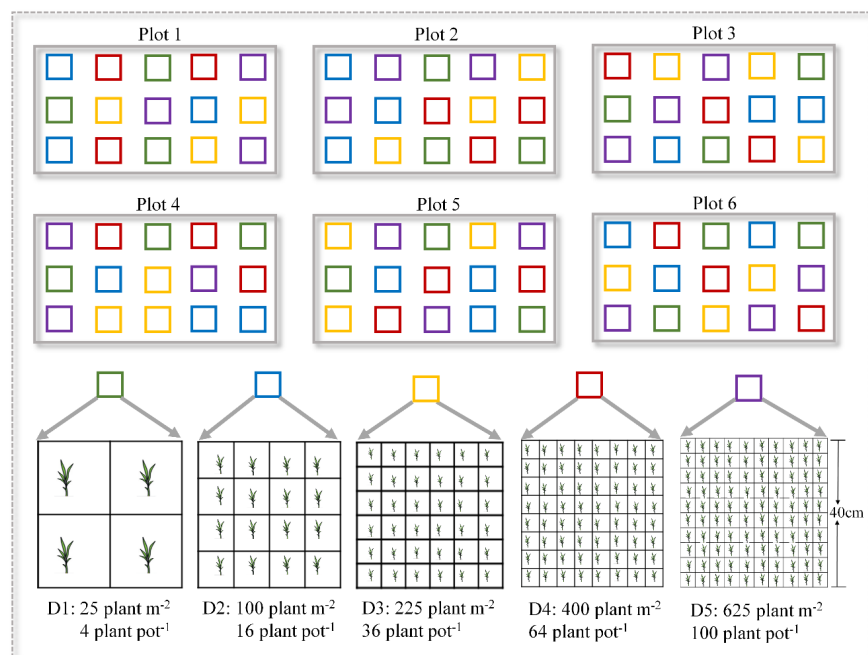


Figure 3

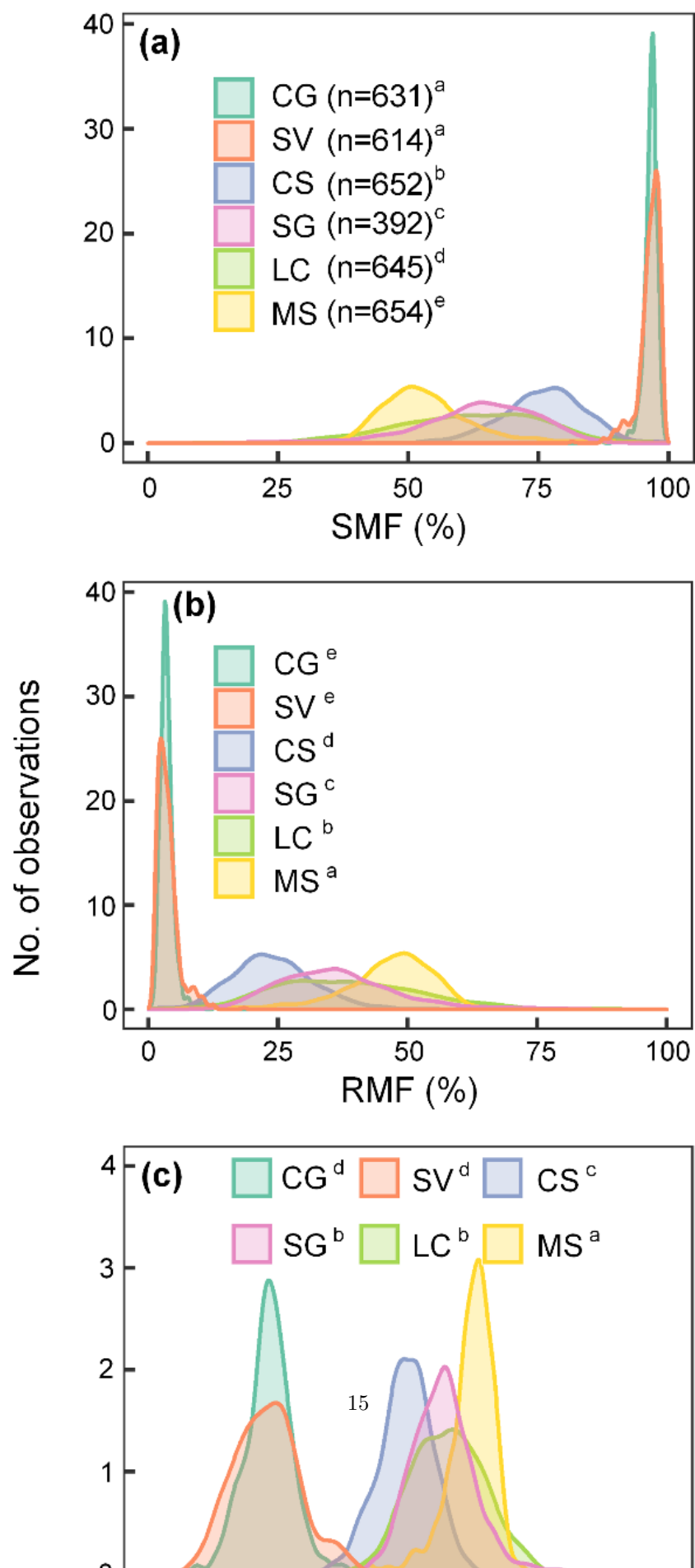


Figure 4

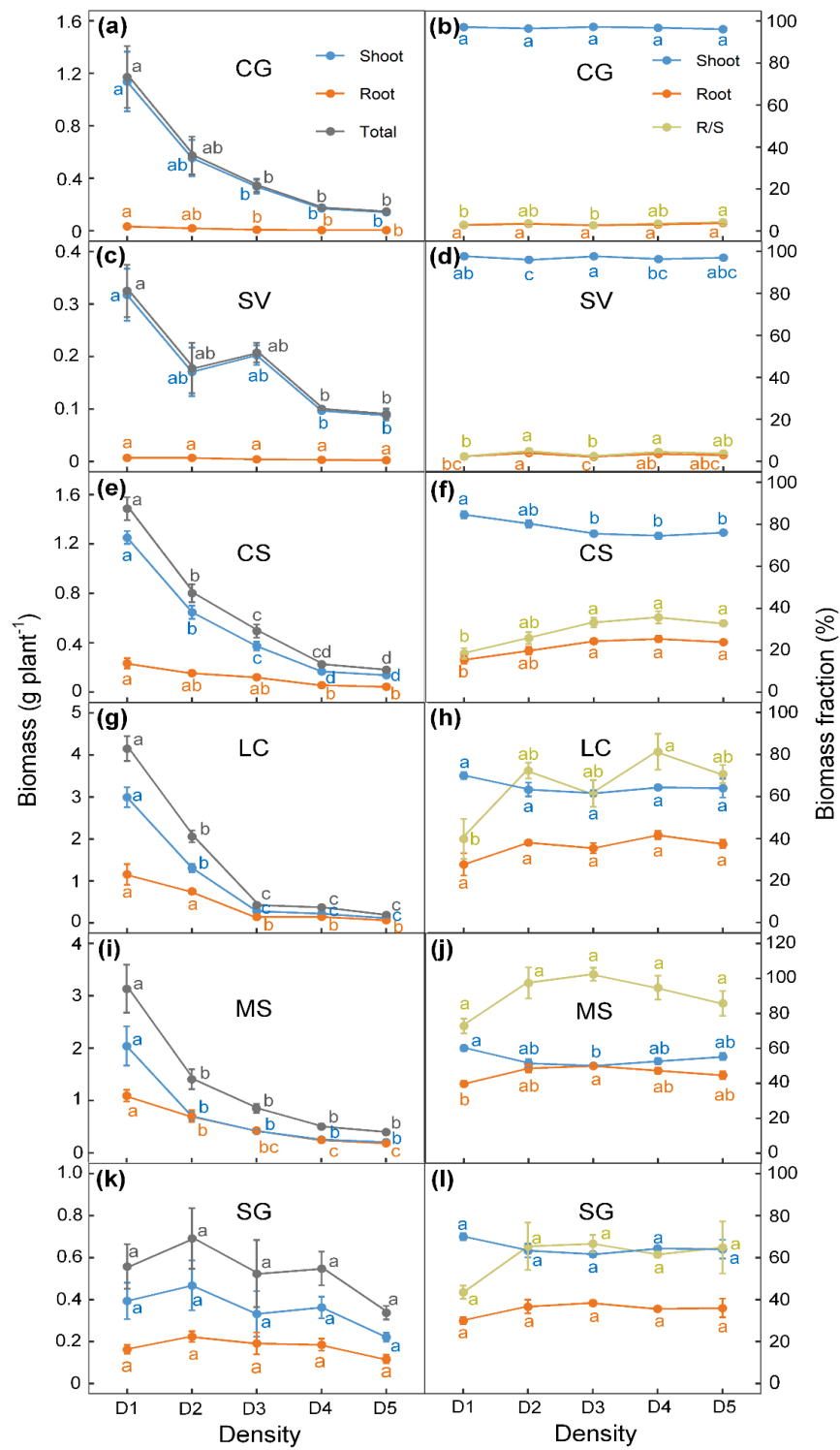


Figure 5

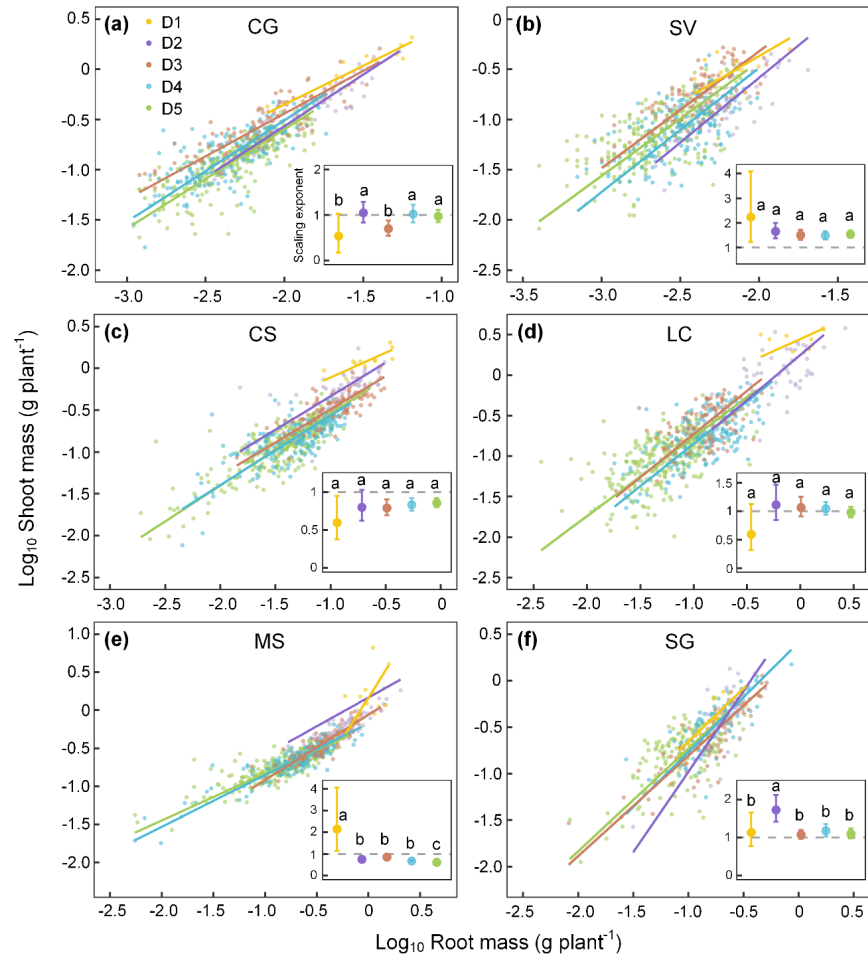


Figure 6

