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Effects of sample size and position from monolith and core methods on the estimation of total root biomass in a temperate grassland ecosystem in Inner Mongolia

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ABSTRACT

Development of an appropriate scheme to accurately sample root biomass is essential for accurate estimation of biomass and carbon budget of grassland. This study evaluated measurement accuracy of the monolith and core methods with different sample sizes and positions in a temperate grassland ecosystem in Inner Mongolia, China. The results indicated that the small core method (3.8-cm-diameter) significantly underestimated total root biomass compared with the large core method (10-cm-diameter), small monolith method (0.25 m^2) and large monolith method (1 m^2). Total root biomass estimated from the small core method was about 52% less than that from the large monolith method (1 m^2). At 95% confidence interval, 10% relative precision could be obtained with five small monoliths, 15 large cores and 65 small cores. The coefficient of variation (CV) for total root biomass decreased logarithmically with increasing sample size for both the monolith and core methods. Compared with the stratified random sampling, core sampling with different fixed positions could not provide reliable estimate of total root biomass. Washing damage and soil lost during extraction might be the major factors controlling the measurement accuracy of total root biomass by core method with small sample size.

1. Introduction

As a critical component of the global carbon cycle, grassland soils store at least 10% of global carbon stocks (Eswaran et al., 1993; Scurlock and Hall, 1998; Prentice et al., 2001). Despite the well-demonstrated distribution pattern of aboveground biomass in terrestrial ecosystems and the importance of fine roots in nutrient cycling, resource capture and global biogeochemistry (Jackson et al., 1997; McNaughton et al., 1998; Bardgett et al., 2005), the belowground biomass still remains as the hidden half of terrestrial ecosystems (Jackson et al., 1996; Waisel et al., 2002). Due to methodological difficulties associated with observing and measuring belowground biomass, our knowledge of root distribution and belowground processes is far from adequate (Vogt et al., 1995; Hendricks et al., 2006).

Many studies have compared the measurement accuracy of root biomass or root distribution from different methods including core method, ingrowth cores, monolith method, minirhizotrons, trench profile wall and core break methods (Majdi et al., 1992; Heeraman and Juma, 1993; Samson and Sinclair, 1994; Vogt et al., 1998; Park et al., 2007). Among those methods, monolith and core methods are verified to give reliable estimates of root biomass and root length density (RLD) despite destructive sampling and high labor requirement (Böhm, 1979; Kücke et al., 1995; Machado and Oliveira, 2003).

Sampling strategy in terms of sample size and sample position may have important consequences for sample variance (Rossi and Nuutinen, 2004). However, the importance of designing an appropriate scheme to accurately sample root biomass has largely been ignored (Van Noordwijk et al., 1985; Bengough et al., 2000). There is as yet no standard soil sample size for the quantification of root biomass. The sample size for monolith method ranges from 0.3 m^2 to 1.2 m^2 (Bormann et al., 1993; Heijmans et al., 2001), and the core diameter used by different investigators varies from 1.9 cm to 15 cm (Vogt and Persson, 1991; Neill, 1992; Hungate et al., 1997; Wilsey and Polley, 2006; Armitage and Fourqurean, 2006). Different sampling designs including stratified random sampling, simple random sampling and systematic sampling are favored by ecologists (Fortin et al., 1989; Brus and De Gruijter, 1997; Klironomos et al., 1999). However, there is

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considerable debate about which scheme is optimal. Oliveira et al. (2000) suggested that a completely randomized design should be used when core samples are taken in grassland or other ecosystems where plants are not grown in rows. Klironomos et al. (1999) found that stratified sampling design would be more efficient than fully randomized design for belowground field studies. Due to the lack of subjective selection criterion, the sample schemes taken by different researchers are either random sampling (Weltzin and McPherson, 2000; Baer et al., 2002; Sierra et al., 2003; Gross et al., 2008) or systematic sampling (Armitage and Fourqurean, 2006; Park et al., 2007). Therefore, it is impossible to compare root biomass measured by different methods or obtained from different studies (Pierret et al., 2005). The best way to assess the reliability of different methods is to apply them simultaneously in the same site, and to compare the results quantified from different methods with the values calculated from a widely accepted method (Hertel and Leuschner, 2002).

In this study, we addressed the issue of measurement accuracy of total root biomass in a semi-arid *Stipa krylovii* grassland with different sample positions by core method with two inner diameters (3.8 cm and 10 cm) and monolith method with two sample sizes (1 m^2 and 0.25 m^2). Based on the simultaneous measurements of total root biomass from two kinds of methods at the same site, we aimed to: (1) investigate the applicability of monolith and core methods for quantifying total root biomass; (2) examine the effect of sample size on the measurement accuracy of total root biomass from monolith and core methods when the spatial distribution of roots was considered; and (3) compare the measurement precision of total root biomass between stratified random sampling and systematic sampling when core method was used.

2. Materials and methods

2.1. Study site

The study was conducted at Xilingol Grazing and Meteorological Station in Inner Mongolia Autonomous region, China (43°08′03″N, 116°19′43″E, 1030 m a.s.l.). This region is described as a semi-arid continent climate with low temperature and limited precipitation. The mean annual temperature and precipitation from 1970 to 2000 are 2 °C and 290 mm. The soil is classified as chestnut soil according to the Chinese Soil Classification System, which is equivalent to calcic–orthic aridisol in the United States Soil Taxonomy (Soil Survey Staff, 2006), with 63% sand, 20% silt, and 17% clay, respectively. The total soil organic matter content in the top 30 cm of soil without roots is 2%–4%. Lime is accumulated below the depth of 40 cm.

The grassland is dominated by cool season C_3 grasses *S. krylovii* and *Leymus chinensis* (Wang et al., 2008), which produce 70% of aboveground biomass, and other species including *Koeleria cristata*, *Carex duriuscula*, *Artemisia frigada*, *Allium mongolicum*, *Cleistogenes squarrosa*, and *Salsola collina*. This study site has been fenced since 1996 and never under any management scheme. It has flat topography and uniform vegetation distribution. Average canopy height is 35 ± 5 cm (mean $\pm$ standard deviation).

2.2. Experimental design

Total root biomass was sampled in September, 2006. Three 1 m × 1 m plots were selected randomly at an interval of 10 m from west to east in the site, and these plots were labeled as 1, 2, and 3. The three plots randomly selected were visually observed to contain similar species composition and uniform growth during the sampling period. Each plot was further divided into four 0.25 m^2 subplots, and these subplots were labeled as 1-1, 1-2, 1-3, 1-4, 2-1, 2-2, 2-3, 2-4, 3-1, 3-2, 3-3, and 3-4, respectively. The aboveground biomass was measured by clipping species at the soil surface in each plot. Total root biomass was then sampled to a depth of 30 cm in 10 cm increments because 83% of roots occurring in this layer of soil (Jackson et al., 1996). There had just been a rain before sampling, so the surface soil (0–20 cm) was moist with loose structure.

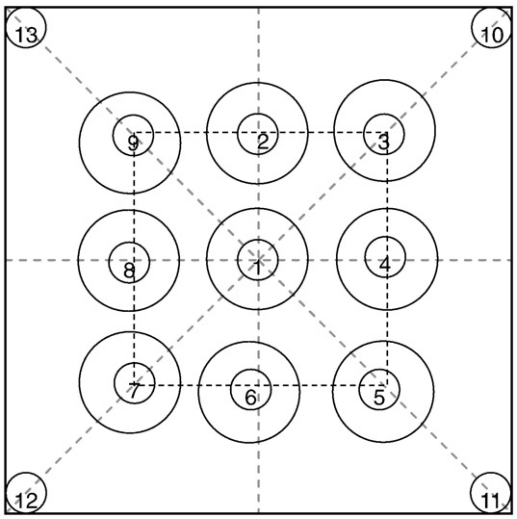

Fig. 1. Arrangement of sample positions for total root biomass from the large core and small core methods in each subplot. Small cores (3.8-cm-diameter) were taken at the positions numbered as 1 to 13 in the figure; large cores (10-cm-diameter) were taken at the positions numbered as 1 to 9 in the figure where small cores had just been taken.

In each subplot (0.25 m^2), firstly, small cores were taken at the positions numbered as 1 to 13 in Fig. 1 using a 3.8-cm-diameter metal auger. When samples were taken on the boundary between two subplots, soil cores were extracted relatively away from the boundary. Secondly, large cores were taken by a 10-cm-diameter metal auger at the positions numbered as 1 to 9 in Fig. 1 where small cores had just been taken. Because of the loose soil structure, particular attention was paid to avoid soil collapse when removing from each core sample. The auger consisted of a cylindrical tube 15 cm high and 10 cm intervals were marked on the outside of the tube and the shaft. The cutting edge of the cylindrical tube was serrated (Böhm, 1979; Vogt and Persson, 1991). Finally, small monolith was excavated from the topsoil down to 30 cm depth, where large cores and small cores had just been taken. One large monolith consisted of four small monoliths.

All the samples were washed separately through a graded series of screens with 2.0, 1.0, and 0.5 mm apertures. This process was repeated several times for all the soil samples to maximize the retrieval of total root biomass. During the washing procedure, a gentle stream of water was added on top of the sieves to facilitate the removal of soil particles, and the roots were separated from the mineral soil and organic matter. Root samples were then oven-dried at 70 °C for 24 h and weighed (Bledsoe et al., 1999).

Soil masses were extracted by core method with three diameters (3.8, 5.05, and 10 cm) at three successive depths (10, 20, and 30 cm) in July 2009. Three replicates were sampled for each treatment. These samples were oven-dried at 105 °C for 48 h and weighed. The values of soil bulk density calculated from core method with three diameters were then compared to investigate the soil compaction resulting from the core method.

2.3. Data analysis

Because of the difficulties in separating live from dead roots and the ambiguous definitions of root death (Bledsoe et al., 1999; Comas et al., 2000; Sierra et al., 2003), total root biomass was considered in this study, making no distinction between live and dead roots (necromass). Total root biomass measured by the large core method was the sum of labeled samples obtained by the large core and small core methods. As for the large monolith method, total root biomass was calculated as the sum of all the labeled samples obtained by the

large core, small core and small monolith methods in this area. In order to investigate the vertical root distribution among soil layers, root mass density (RMD) was calculated by the large core method based on the large core volume (785 cm^3).

Two sampling schemes were used to measure total root biomass. For the first scheme, core samples were taken under stratified random sampling. For monolith method, there were four equal subplots in each 1 m^2 plot. We randomly chose one, two, and three subplots from each plot according to the random permutation of the four subplots. There were four options when one small monolith was taken in each plot, and the number of options changed to six and four when two and three monoliths (0.25 m^2) were taken according to the permutation of four samples. We took the average value of two or three samples as total root biomass for each permutation. As for the large core method, at first one core was chosen randomly from nine samples, there were nine options. When the number of core samples chosen from each subplot ranged from 1 to 8, the number of options changed accordingly with the permutation of nine samples. For the small core method, we randomly chose one to 12 core samples from 13 samples. There were 13 and 78 options when one and two core samples were chosen from 13 samples according to the random permutation of 13 samples. For the second sampling scheme, several systematic arrangements of sample positions were compared in this study, including one core at the center, five cores with one center and four corners, and nine cores with one center and eight corners. These arrangements had been widely used for sampling total root biomass in grassland ecosystems (Craine and Wedin, 2002; Armitage and Fourqurean, 2006; Liao and Boutton, 2008).

Coefficient of variation (CV) was used to detect the sample variance caused by spatial heterogeneity of vegetation in the study site.

$$CV = Std / Mean \times 100 \quad (1)$$

where Std and Mean represent the standard deviation and the mean of samples.

The number of replicates (n) could be determined by the following equation proposed by Krebs (1999):

$$n = (100 CV t_\alpha / r)^2 \quad (2)$$

where t_α is the Student's t value with $n-1$ degrees of freedom for $1-\alpha$ level of confidence, and r is the desired relative precision. In this study, α was set to 0.05, which meant that the probability of making a type I mistake was 5%; the desired relative precision (r) was set to 10, which indicated that the sampling error was required within $\pm 10\%$ relative precision.

2.4. Statistical analysis

All the statistical analyses were carried out using SPSS 11.5 for Windows (SPSS Inc, Chicago, Illinois). The Shapiro–Wilk normality test was used to check the normality of data and Log_{10} transformation was used to normalize data that were not normally distributed. One-way analysis of variance (ANOVA) and Fisher's LSD multiple comparisons were used to compare the measurement accuracy of the large monolith, small monolith, large core and small core methods and to compare the soil bulk density calculated from core method with three diameters. Moreover, nonlinear regression analysis was used to determine the effect of sample size on sampling error. In all cases, a level of P less than 0.05 was accepted as statistically significant.

3. Results

3.1. Effect of sample size on measurement accuracy of total root biomass

Total root biomass quantified by the large monolith method averaged 3794 ± 84 g m^{-2} across all three plots (Table 1), and was considered to be true value of total root biomass to evaluate the measurement accuracy of other methods. The one-way ANOVA analysis showed that total root biomass in soil layers of 0–10 cm ($F_{3,8}=15.92$, $P=0.001$), 10–20 cm ($F_{3,8}=39.58$, $P<0.001$), 20–30 cm ($F_{3,8}=7.39$, $P=0.01$), and 0–30 cm ($F_{3,8}=47.63$, $P<0.001$) were all significantly different among four methods. Compared with the large monolith method, both the small monolith method and large core method underestimated total root biomass for all the soil depths. However, there was no significant difference. The small core method underestimated total root biomass significantly for all the soil depths compared with the large core method and two monolith methods, respectively ($P<0.01$). The root mass density was concentrated in the top 10 cm of soil, which comprised 58% of total root biomass, and exhibited sharp decrease with soil depth. The root mass density was 20, 8, and 6 mg cm^{-3} at 0–10, 10–20, and 20–30 cm soil depths, respectively.

Table 1
Measurement accuracy of total root biomass (live plus dead) from monolith and core methods with different sample sizes in the temperate grassland ecosystem.

Sample method	Total root biomass(g m^{-2})			
	0–10 cm	10–20 cm	20–30 cm	0–30 cm
Monolith method (1 m^2)	2236 (193)[a]	970 (69)[a]	588 (57)[a]	3794 (84)[a]
Monolith method (0.25 m^2)	2137 (137)[a]	932 (35)[a]	563 (56)[a]	3632 (65)[a]
Large core method (10-cm-diameter)	2006 (30)[a]	855 (47)[a]	594 (57)[a]	3454 (34)[a]
Small core method (3.8-cm-diameter)	1179 (37)[b]	317 (35)[b]	315 (21)[b]	1811 (243)[b]

Values were the mean (standard error) of three replicate samplings for the large monolith, small monolith, large core and small core methods, respectively. Means with different letters identify significant differences ($P<0.05$) among different methods for each variable using one-way ANOVA (Games–Howell post hoc test) and Fisher's LSD multiple comparisons.

The coefficient of variation (CV) for total root biomass increased consistently with increasing soil depth for the small monolith method (Table 2). This was mainly due to the difficulty in digging soil samples out as sampling depth increased. However, the large core method was insensitive to sampling depth. The maximum sampling error appeared at 0–10 cm soil depth for both the large core and small core methods. The values of CV for total root biomass at 0–30 cm soil depth were smaller than those in each 10 cm interval soil layer for both the monolith and large core methods.

3.2. Effect of sample size on measurement precision of total root biomass from stratified random sampling

The measurement precision of total root biomass increased with increasing sample size for all the sampling methods at three soil depths. The decrease in CV for total root biomass (%) could be described as a logarithmic function of sample size (m^2) as follow (Fig. 2):

$$CV = 14.37 - 3.42 \ln(sample\ size - 0.0011) (R^2 = 0.75, n = 26, P < 0.001) \quad (3)$$

Thus, the number of replicates (n) could be obtained by:

$$n = (100(14.37 - 3.42 \ln(sample\ size - 0.0011)) t_\alpha / r)^2 \quad (4)$$

In order to obtain the same margin of error with the variance observed, when one sample was taken in each subplot, the number of replicates required would be 5, 15, and 65 for the small monolith, large core, and small core methods, respectively.

Table 2
Effects of sample size on measurement precision of total root biomass from the monolith method, large core and small core methods by stratified random sampling.

Sample method	Sample size (m^2)	Sample number in each subplot (n)	Coefficient of variation for total root biomass (%)			
			0–10 cm	10–20 cm	20–30 cm	0–30 cm
Monolith method (0.5 m^2)	0.25	1	14	17	17	11
	0.5	2	8	13	15	6
	0.75	3	5	11	14	4
	1	4	3	12	17	3
Large core method (10-cm-diameter)	0.0054	1	31	30	30	20
	0.0107	2	25	24	23	16
	0.0160	3	23	21	21	14
	0.0214	4	22	20	19	14
	0.0268	5	21	19	19	13
	0.0321	6	21	18	18	13
	0.0374	7	21	18	18	12
	0.0428	8	21	18	17	12
	0.0482	9	21	18	18	13
Small core method (3.8-cm-diameter)	0.0011	1	55	44	49	41
	0.0022	2	43	34	38	33
	0.0033	3	38	30	34	30
	0.0044	4	36	28	31	28
	0.0055	5	34	27	30	27
	0.0066	6	33	26	29	27
	0.0077	7	32	25	28	26
	0.0088	8	31	24	27	26
	0.0099	9	31	24	27	25
	0.0110	10	30	24	27	25
	0.0121	11	30	23	26	25
	0.0132	12	29	23	26	25
	0.0143	13	31	24	27	26

3.3. Effect of sample position on measurement precision of total root biomass from systematic sampling

As for the systematic sampling, the sampling error did not show any regular change among different sample sizes, sample positions and sample depths. There was enormous variation in the sampling error among different sample positions with the same sample size, especially for the small core method (Table 3). When five small cores were taken from each subplot, the CV for total root biomass at 20–30 cm soil depth was 37% at position 1-2-4-6-8, which was much higher than that at the position 1-3-7-10-12 (26%).

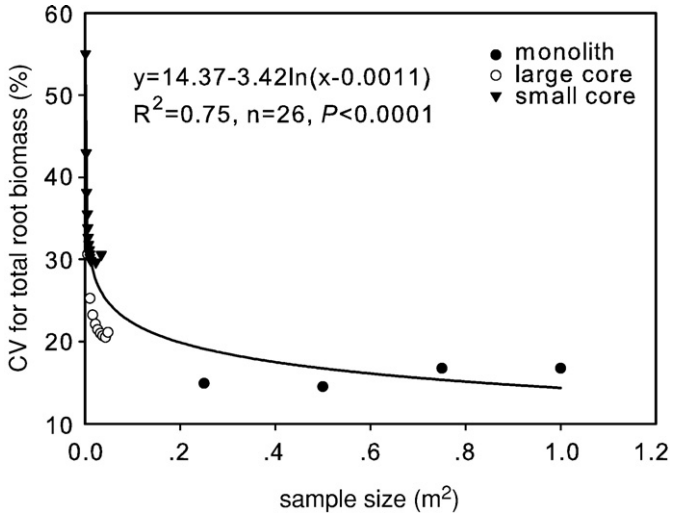

Fig. 2. Effect of sample size on coefficient of variation (CV) for total root biomass in the temperate grassland ecosystem. Values were the maximum CV for total root biomass in three soil layers from the monolith, large core (10-cm-diameter) and small core (3.8-cm-diameter) methods.

When only one sample was taken at the center of the subplot, the maximum CV for total root biomass was 31% and 65% at 20–30 cm soil depth for the large core and small core methods, respectively. Compared with the values of CV for total root biomass from random sampling with the same sample size, the systematic sampling with one center and four corners or 'Z' shape sampling for the large core method and all the sampling positions for the small core method (except position 1-10-11-12-13) could not reduce the sampling error.

4. Discussion and conclusions

4.1. Effect of sample size on estimation of total root biomass

It would be convenient to compare different root sampling methods if one method was known to be the most accurate. Because of the large sample area, the large monolith method was always considered as the standard method (Park et al., 2007). Compared with the standard method, the small monolith method, large core and small core methods all underestimated total root biomass in this temperate grassland ecosystem. This result was consistent with the observations in other ecosystem (Sun et al., 1994). To a certain extent, such underestimation might be caused by washing damage and soil lost during the extraction of total root biomass. Washing procedure underestimated 30% or more of fine root biomass in a tropical forest ecosystem (Sierra et al., 2003). In this study, washing damage resulted in approximately 5% underestimation of total root biomass per square meter for the small monolith method. Compared with the large monolith method, the sample size of large core and small core methods decreased 184 and 880 times, respectively. The amount of roots in large core and small core were low and the fraction of roots lost during washing procedure would be relatively high. When it converted to total root biomass per square meter, the washing damage would be even larger relative to that from the large monolith method. It would have a relatively larger impact on the small core method than on the large core. Our results suggest that for each method, all the samples should be washed and weighed together to

Table 3
Coefficient of variation (CV) for total root biomass from the large core and small core methods sampled by systematic and random samplings based on the same sample size.

Sample method	Sample number in each subplot (n)	Position arrangement	CV for total root biomass (%)			
			0–10 cm	10–20 cm	20–30 cm	0–30 cm
Large core method (10-cm-diameter)	1	1	29 (31)	22 (30)	31 (30)	20 (19)
	5	1-3-5-7-9	22 (21)	20 (19)	21 (18)	14 (13)
		1-2-4-6-8	22 (21)	18 (19)	16 (18)	12 (13)
	7	1-2-3-5-6-7-9	23 (21)	21 (18)	20 (18)	14 (12)
		1-3-4-5-7-8-9	20 (21)	17 (18)	18 (18)	12 (12)
Small core method (3.8-cm-diameter)	1	1	41 (55)	48 (37)	65 (49)	30 (37)
	4	2-4-6-8	37 (36)	31 (27)	30 (32)	32 (28)
		3-5-7-9	41 (36)	27 (27)	30 (32)	30 (28)
		10-11-12-13	38 (36)	25 (27)	27 (32)	28 (28)
	5	1-2-4-6-8	30 (34)	29 (26)	37 (30)	26 (27)
		1-3-5-7-9	32 (34)	25 (26)	33 (30)	25 (27)
		1-10-11-12-13	29 (34)	25 (26)	26 (30)	23 (27)
		1-3-7-10-12	30 (34)	28 (26)	26 (30)	26 (27)
		1-5-9-11-13	30 (34)	20 (26)	35 (30)	22 (27)
	7	1-2-3-5-6-7-9	31 (32)	21 (25)	33 (28)	25 (26)
		1-3-4-5-7-8-9	33 (32)	30 (25)	31 (28)	28 (26)
		1-5-9-10-11-12-13	31 (32)	19 (25)	27 (28)	24 (26)
		1-3-7-10-11-12-13	28 (32)	26(25)	26 (28)	24 (26)
	9	1-2-3-4-5-6-7-8-9	32 (31)	26 (24)	32 (27)	27 (25)
		1-2-4-6-8-10-11-12-13	28 (31)	26 (24)	28 (27)	25 (25)
		1-3-5-7-9-10-11-12-13	32 (31)	22 (24)	26 (27)	24 (25)

The data in the bracket were the coefficient of variation for total root biomass from random sampling with the same sample size. The numbers in position arrangement correspond to those in Fig. 1. Several systematic positions were compared, including one center (1), one center and four corners (1-3-5-7-9 and 1-10-11-12-13), 'Z' shape sampling (1-3-7-10-12, 1-5-9-11-13, 1-5-9-10-11-12-13, and 1-3-7-10-11-12-13), and so forth.

relatively increase the sample area in each subplot and to decrease the fraction of washing damage.

The value of CV for total root biomass decreased logarithmically in response to increasing sample size, which was consistent with the finding of Sun et al. (1994) in the temperate forest–grassland ecosystem, where core method with four diameters (5, 6, 7, and 9 cm) were used for estimating total root biomass of *Bothriochloa ischaemum* grassland (Fig. 3). When compared with the large core and small core methods, larger soil volume sampled by the monolith method might reduce root spatial heterogeneity and therefore reduce the sampling error (Heeraman and Juma, 1993; Kücke et al., 1995).

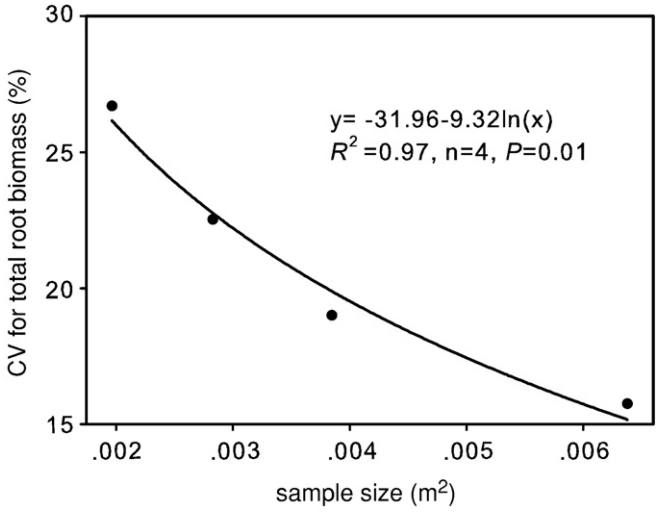

Fig. 3. Relationship between coefficient of variation (CV) for total root biomass and sample size in the forest–grassland ecosystem. Data were obtained from Sun et al. (1994). Core methods with four diameters (5, 6, 7, and 9 cm) were used for quantifying total root biomass in Loess plateau.

The number of samples varies greatly among different ecosystems based on the sample size, local vegetation heterogeneity and soil structure. In this study, 10% relative precision could be obtained at 95% confidence interval with at least five monoliths (0.25 m^2), 15 cores (10-cm-diameter), and 65 cores (3.8-cm-diameter). When it came to Bartlett forest ecosystem, at least 28 cores (5-cm-diameter) or 20 pits (0.5 m^2) were required (Park et al., 2007). Ten cores (9-cm-diameter) were required for *Bothriochloa ischaemum* grassland in Loess plateau to obtain the same margin of error (Sun et al., 1994). However, we always have to balance the measurement accuracy and precision with financial/time availability in practice. Our results indicate that one might not be able to financially take more monoliths but more 'smaller cores' to truly reflect the variability and even average mass of total root biomass.

4.2. Effect of sample location on estimation of total root biomass

Sample location depends on the purpose of the study and the spatial distribution of root system (Bengough et al., 2000). When the aim is to estimate population parameters like mean or variance in the field, stratified sampling is desirable (Fortin et al., 1989; Oliveira et al., 2000). Meanwhile, considering the simplicity of application and the desire to sample evenly across the whole plot, systematic sampling is often used in the field to detect the spatial patterns of soil properties (Bourdeau, 1953; Brus and De Gruijter, 1997; Krebs, 1999; Augustine and Frank, 2001). With the assumption of root distribution, Van Noordwijk et al. (1985) developed the general sampling schemes for a range of ecosystems (e.g. grassland, crops including cereals, sugar beet and potatoes) using a 7-cm-diameter core. Our study site had uniform distribution and similar species composition. The aboveground biomass ranged from 191 to 230 g m^{-2} with 10% CV among three plots and the plants were spaced closely in all the directions, thus, a simple random sampling was recommended. Systematic sampling could not reduce the sampling error compared with random design with the same sample size for the large core and small core methods in this study (Table 3). Furthermore, contrary to random design, there was no decrease in variance of systematic design with increasing sample size. Our result was consistent with the finding reported by Goslee (2006) where

systematic and random samplings were compared for estimating species frequency. Our results suggest that stratified random sampling would be more suitable than systematic design for the measurement of total root biomass in this study site.

4.3. Soil compaction associated with core method

One of the problems relevant to core method is whether it could give reliable measurement of total root biomass (Persson, 1990; Vogt et al., 1998). It is common that core method can cause soil compaction, which can result in overestimation of total root biomass (Vogt and Persson, 1991). Soil compaction would decrease with increasing core diameter and can be handled by using cores with inner diameter larger than 6 cm and with beveled and serrated edge (Campbell and Henshall, 2001; Park et al., 2007). Moreover, as far as the vertical compaction by core method is concerned, one simple way to avoid sampling error is to sample beyond the maximum rooting depth.

Park et al. (2007) found that soil compaction resulted in about 10% overestimation of root biomass. However, both our study and Sun et al. (1994) showed that total root biomass was underestimated by core method with small diameter. Sun et al. (1994) reported that compared with 9-cm-diameter core, 5-cm-diameter core underestimated about 16% of total root biomass. Our results indicated that 3.8-cm-diameter core and 10-cm-diameter core methods underestimated about 53% and 9% of total root biomass compared with the monolith method (1 m^2), respectively. There would be at least two reasons for this difference: (1) this underestimation might be partly attributed to soil lost from core tube. Sun et al. (1994) found that among the 16% underestimation of total root biomass by 5-cm-diameter core, washing damage contributed to about 5% of this difference and the residual 11% was caused by soil lost during the sampling. The soil in the research conducted by Sun et al. (1994) was characterized by loose structure and high erodibility. Similarly, the soil in this study was classified as very loose sands. Therefore, when core samples were taken in these areas, it was inevitable that the soil lost occurred. (2) Different sensitivities to compaction exhibited by different soil types (Whalley et al., 1995; Lipiec and Hatano, 2003; Hamza and Anderson, 2005). The sandy loam soil derived from sandstone was moderately compressible while the loose structure and sandy soil in Sun's and our study area exhibited considerable resistance to compression (Smith et al., 1997; Park et al., 2008). The soil bulk density calculated from core method increased with increasing core diameter at all the soil depths in this study (Table 4). Compacted soil would result in higher soil bulk density. However, compared with the large core method, the small core method significantly underestimated the soil bulk density in all three soil layers, which indicated that the study soil was relatively loose and could not be compacted by the small core method. Therefore, when core sampling was conducted on solid soil with high content of clay plus loam, the soil compaction would probably result in overestimation of total root biomass. In contrast, when core samples were taken from high sand content or loose structure soil, the underestimation of total root biomass would occur due to the soil lost during the extraction process.

Table 4
Comparison of soil bulk density calculated from core method with three diameters in the temperate grassland ecosystem.

Sample method	Soil bulk density (g cm^{-3})		
	0–10 cm	10–20 cm	20–30 cm
Large core method (10-cm-diameter)	1.64 (0.05)[a]	1.64 (0.14)[a]	1.87 (0.15)[a]
Median core method (5.05-cm-diameter)	1.23 (0.03)[b]	1.07 (0.02)[b]	1.24 (0.06)[b]
Small core method (3.8-cm-diameter)	0.88 (0.03)[c]	1.04 (0.07)[b]	1.07 (0.02)[b]

Values were the mean (standard error) of three replicate samples for the large core, median core, and small core methods, respectively. Means with different letters identify significant differences ($P<0.05$) among different methods for each variable using one-way ANOVA (Games–Howell post hoc test) and Fisher's LSD multiple comparisons.

4.4. Recommendations for sampling total root biomass

Based on our results, two sets of recommendations were elaborated when sampling total root biomass in the field:

(1) In order to improve the measurement accuracy of total root biomass from core method, firstly, it is suggested that washing damage should be reduced by washing all the samples together; secondly, when taking core samples from high sand content or loose structure soils, caution must be paid to minimize soil lost during sampling procedure; otherwise, when taking samples from soils with high clay plus loam content according to the literature, core method with larger than 6 cm in diameter should be used to eliminate soil compaction.

(2) In order to improve the measurement precision of total root biomass, the sample size should be determined at the beginning of the study and be controlled to get a reliable measurement of total root biomass. However, in practice, we have to be concerned with the trade-off between statistical precision and financial/time costs.

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