Effects of soil stoichiometry of the CaCO_3 /available phosphorus ratio on plant density in *Artemisia ordosica* communities

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Received August 9, 2011; accepted September 21, 2011; published online December 2, 2011

In soil, CaCO_3 can reduce the availability of phosphorus in arid and semi-arid regions, thereby influencing plant growth. However, the effects of soil stoichiometry of the CaCO_3 /available phosphorus ratio (CaCO_3 /AP ratio) on plant density are unknown. In this study, we examined 4 *Artemisia ordosica* communities located in arid and semi-arid regions, and performed a pot experiment with an orthogonal design to examine the relationships between the soil CaCO_3 /AP ratio and plant density. The results showed that the soil stoichiometry of the CaCO_3 /AP ratio had a greater effect on growth of *A. ordosica* than either CaCO_3 or AP as single factor. The influence of the soil CaCO_3 /AP ratio on density of *A. ordosica* was related to the climatic zone and the types and/or amplitudes of increases in the soil CaCO_3 /AP ratio. When the soil CaCO_3 /AP ratio was within the same amplitude and was less than 2.5, the *A. ordosica* density increased in the semi-arid region, whereas the density decreased with increasing soil CaCO_3 /AP ratios in the arid region. In the semi-arid region, when the soil CaCO_3 /AP ratio was within the same amplitude and the increase in the soil CaCO_3 content was greater than the increase in soil AP, the *A. ordosica* density increased with increasing soil CaCO_3 /AP ratios. This means that the relationship between the soil CaCO_3 /AP ratio and the *A. ordosica* density remained the same. In the arid region, an increase in the soil CaCO_3 /AP ratio as a result of increased soil CaCO_3 content and decreased soil AP resulted in increased density of *A. ordosica*. Therefore, the relationship between soil CaCO_3 /AP ratios and density of *A. ordosica* was altered by these changes in soil chemistry. Thus, the soil stoichiometry of the CaCO_3 /AP ratio is an important factor affecting the density of *A. ordosica* in arid and semi-arid regions.

***Artemisia ordosica*, soil CaCO_3 , soil available phosphorus, soil CaCO_3 /available phosphorus ratio (soil CaCO_3 /AP ratio), stoichiometry, plant density**

Citation: Zhao X L, He X D, Xue P P, et al. Effects of soil stoichiometry of the CaCO_3 /available phosphorus ratio on plant density in *Artemisia ordosica* communities. Chin Sci Bull, 2012, 57: 492–499, doi: 10.1007/s11434-011-4866-4

Ecological stoichiometry is a new research field for ecology [1–3]. During the process of asexual- and sexual-reproduction of organisms, variations in soil stoichiometry can lead to changes in biological stoichiometry, which can influence interspecies relationships within plant communities in various ecosystems with different energy inputs and nutrient supplies [4]. Soil stoichiometry and its dynamics, therefore, are important factors to take into account when evaluating limiting elements, nutrient use efficiency, population dynamics,

and community succession [5,6]. Studies on ecological stoichiometry provide a new way to study the interactions between soil and plants.

In arid and semi-arid regions, soil CaCO_3 and available phosphorus (AP) are important factors for plant growth [7–9]. However, soil CaCO_3 readily immobilizes AP as a result of adsorption and precipitation [10,11]. Higher levels of CaCO_3 in the soil greatly decrease phosphorus availability, which strongly affects plants in arid and semi-arid regions because there is little precipitation [10,12,13]. The phosphorus limitation will then affect plant growth and even

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plant density within communities. As stated in Tilman's resource-ratio hypothesis, when the relative availability of limiting resources changes, plant species will specialize to secure different proportions of limiting resources, and then the composition of the plant community, i.e., plant density, will change [14]. However, at present, it is unknown whether plant density is affected by the soil stoichiometry of the CaCO_3/AP ratio, which reflects the degree of immobilization of AP by CaCO_3 .

Theoretical analyses indicate that the soil stoichiometry of the CaCO_3/AP ratio will influence plant growth in arid and semi-arid regions. It will initially influence plant biomass, and then affect plant density. For this study, we selected *A. ordosica* as the test species, and investigated four *A. ordosica* communities located in arid and semi-arid regions. We performed a pot experiment with an orthogonal design to examine the relationships between the soil CaCO_3/AP ratio and plant density. Our objectives were as follows: (1) to determine whether soil stoichiometry of the CaCO_3/AP ratio is a factor influencing plant density; and (2) to determine how plant density responds to different types/amplitudes of changes in the soil stoichiometry of the CaCO_3/AP ratio.

1 Materials and methods

In this study, we carried out field surveys and a pot experiment from 2004 to 2011.

1.1 Field studies

We selected 4 sample plots for detailed surveys: a one-dimensional sample plot in an *A. ordosica* community on a fixed dune in Shapotou, Ningxia Hui Autonomous Region; a one-dimensional plot in an *A. ordosica* community on a semi-fixed dune and a two-dimensional plot in an *A. ordosica* community on a fixed dune in Yulin, Shaanxi Province; and a two-dimensional plot in an *A. ordosica* + *A. frigida* community on an overgrown sand dune in Alxa, Inner Mongolia Autonomous Region. Here, the definitions of fixed dune, semi-fixed dune, and overgrown sand dune are as described by Zhu [15] and Zhao [16].

The one-dimensional plot in Shapotou is in the artificial vegetation area built in 1974 at the Research Station of Chinese Academy of Sciences located on the south-eastern boundary of the Tengger Desert, located at $37^\circ 32' 10''\text{N}$, $105^\circ 02' 07''\text{E}$, in an arid area. The average annual air temperature is 9.6°C . The average annual precipitation is 186.6 mm and average annual evaporation is 3000.7 mm. The Aeolian landform type here is checkerboard sand dune. The community is dominated by *A. ordosica*, with a few individuals of *Bassia dasphylla*, *A. scoparia*, *Eragrostis poaeoides*, and others.

The one-dimensional and two-dimensional plots in Yulin

are both in the Nansha air-seeding afforestation area in Qinhe, a town located on Mu Us Sand Land, located at $38^\circ 17' 37''\text{N}$, $109^\circ 40' 14''\text{E}$, which lies in a semi-arid area. The average annual air temperature is 8.1°C . The annual precipitation is 414 mm and annual evaporation is 1905 mm. The Aeolian landform type in this area is faveolate semi-fixed and fixed sand dune. In the two sample plots, the plant communities are dominated by *A. ordosica*, with a few individuals of *Agriophyllum squarrosum*, *Psammochloa villosa*, *Corispermum mongolicum*, *Salsola collina*, and others.

The two-dimensional plot in Alxa is 5-km away from the Alxa Left Banner on the eastern boundary of Tengger Desert, which is located at $38^\circ 24' 36''\text{N}$, $105^\circ 43' 18''\text{E}$, in an arid area. The average annual air temperature is 7.6°C . The average annual precipitation is 213.1 mm and average annual evaporation is 2349.2 mm. The community is in succession, and *A. frigida* is replacing *A. ordosica*. Co-occurring species in this community include *E. poaeoides*, *Ferula bungeana*, *A. scoparia*, *Cleistogenes squarrosa*, *Stipa glareosa*, *Calligonum mongolicum* and others.

In July, 2004, we established one-dimensional sample plots in *A. ordosica* communities on the semi-fixed dune of Yulin and the fixed dune of Shapotou. In each sample plot, we laid out a 500-m sample line in an east-west direction, and then placed $4\text{ m} \times 4\text{ m}$ quadrats at 10-m intervals along the sample line, giving a total of 50 quadrats per sample line. In each quadrat, we recorded plant species and abundance, and collected mixed soil samples from the 0–20 cm layer. The soil samples were taken back to the laboratory for analysis.

In September, 2009, we established two-dimensional plots in the *A. ordosica* + *A. frigida* community on the overgrown sand dune of Alxa and in the *A. ordosica* community on the fixed sand dune of Yulin. Each sample plot covered an area of $40\text{ m} \times 40\text{ m}$, comprising 100 quadrats of $4\text{ m} \times 4\text{ m}$. We recorded species and abundance in each quadrat, and collected mixed soil samples from the 0–20 cm layer for analysis. The samples were collected from the 0–20 cm layer because the calcic horizon has formed at 20–40 cm depth in Alxa, as determined in our previous study [17].

1.2 Pot experiment

We performed a pot experiment with an orthogonal design in a greenhouse at the College of Life Sciences, Nankai University. The orthogonal array was $L_{16}(4^5)$ with 3 replicates for each treatment. The 4 levels of soil CaCO_3 were 1, 5, 25 and 100 g kg^{-1} soil, and the 4 levels of soil AP were 0.1, 3, 6 and 12 mg kg^{-1} soil. The 4 irrigation levels were 200, 250, 300, and 350 g water at each irrigation time. We aimed to examine the effects of the soil CaCO_3/AP ratio within three factors.

The seeds of *A. ordosica* used for the pot experiment were collected from Alxa in the Tengger Desert. The sand matrix used in the pot experiments was also obtained from

the Tengger Desert. The *A. ordosica* seeds were sown on 15 October 2010 into substrate (turf soil:perlite=1:1, v/v) in PVC containers. Three or 4 seeds were sown in each container and were covered with a thin paper film. The paper film was removed after germination and the soil mix was kept moist.

The experimental treatments commenced on December 15, 2010. According to the treatment levels in the experimental design, we added appropriate amounts of CaCO_3 and phosphorus to separate portions of sand, mixed each portion well, and then used them to fill numbered PVC pots (195 mm high, 165 cm in diameter). The soil in each pot was 12 cm deep and 2 kg in weight. Seedlings were transplanted into the pots (one seedling/pot). During this process, we divided the *A. ordosica* seedlings into 3 size classes: larger, medium, and smaller, and each treatment included all 3 sizes as the 3 replicates. After transplantation, seedlings were watered with 400 mL distilled water per pot. This experiment consisted of 48 pots in total.

We measured the heights of 48 *A. ordosica* seedlings on December 20, 2010, and then marked each seedling with red paint at the base of the stem so that plant height could be measured accurately after the experiment. On the same day, the 50 remaining seedlings were dug up and cleaned, and their aboveground and root biomasses, plant height, and root length were measured. The plants in the pot experiment were watered with distilled water once a week. The water was applied via a metal needle to 2-cm below the soil surface to prevent evaporation. During the experiment, the temperature in the greenhouse was kept at approximately 25°C during the day and 18°C at night.

The pot experiment ended on January 30, 2011, after the *A. ordosica* seedlings had grown for 40 d. The 48 seedlings were dug up and cleaned, and then aboveground and root biomasses, plant height, and root lengths were determined.

1.3 Soil sample analyses

In the laboratory, the soil samples were air-dried, ground, and then sieved through an 80-mesh sieve. We used the aerometric method [18] to determine CaCO_3 content, and AP was determined with 0.5 mol L^{-1} NaHCO_3 extraction and the molybdenum stibium anti-color method.

1.4 Data analyses

(i) Calculation of growth rate. Using plant height as the independent variable and plant root length, aboveground biomass, and root weight as dependent variables, we constructed a fitting curve using SPSS 13.0 software and chose the optimal regression equation that showed the best fit to the 50 seedlings measured at the start of the experiment. Then, using the initial plant heights of 48 *A. ordosica* seedlings measured previously as independent variables, we used the optimal regression equation above to estimate the

initial growth status of root length, aboveground biomass, and root weight of these 48 seedlings at the start of the experiment. Thus, the initial plant heights measured previously, the estimated aboveground biomass, root length and root weight served as baseline data for the start of the experiment. Lastly, we calculated the amount of growth using the initial baseline data and the measured data at the end of the experiment, and growth rates were calculated by dividing the amount of growth by the experimental growth period (40 d).

(ii) Data analysis. Using soil nutrient as the horizontal ordinate and plant density as the vertical ordinate, we carried out regression analyses for all of the variables using Excel 2003 software. The variable distribution diagrams were constructed with Surfer 6.0 software and the descriptive statistics and the significance tests were performed using SPSS 13.0 software.

2 Results and analysis

2.1 Variations in soil stoichiometry of the CaCO_3 /AP ratio in four sample plots of *A. ordosica* communities

Before analyzing the effects of soil stoichiometry of the CaCO_3 /AP ratio on plant density, we characterized the soil CaCO_3 /AP ratios (magnitudes and variations) in the study areas.

In the two-dimensional sample plots in Yulin, soil CaCO_3 /AP ratios varied with the terrain of the fixed sand dune. This was because the sand dunes were fixed only for a short time and because the distribution of *A. ordosica* plants was affected by the terrain of the dune. The soil CaCO_3 /AP ratios were relatively higher in the west of the sample plot, and relatively lower in the center and east of the plot (Figure 1(a)). However, apart from a small area in the southwest of the plot, the CaCO_3 /AP ratios were reasonably consistent within the plot in the overgrown sand dune of *A. ordosica* + *A. frigida* community in Alxa (Figure 1(b)). This was because the plant community in this area was undergoing succession. In this plot, the spatial distribution characteristics of soil CaCO_3 /AP ratios would affect the distribution of the various plant species.

Theoretically, there are 3 ways in which the soil stoichiometry of the CaCO_3 /AP ratio can increase. First, the CaCO_3 /AP ratio can increase because of increased CaCO_3 and decreased available phosphorus; second, it can result from the amplitude of the increase in CaCO_3 being greater than that of the available phosphorus; third, it can result when the decrease in soil CaCO_3 is less than the decrease in soil available phosphorus.

Descriptive statistics for one-dimensional sample plots indicated that the CaCO_3 /AP ratio in the semi-fixed sand dune *A. ordosica* community in Yulin (0.43) was less than that in Shapotou (1.08) (Table 1). According to the mean and coefficients of variation of these two plots, the higher

soil CaCO_3/AP ratio in the fixed sand dune in Shapotou was because the soil CaCO_3 increased while AP decreased.

Descriptive statistics for two-dimensional sample plots (Table 2) indicated that the soil CaCO_3/AP ratio in the fixed sand dune *A. ordosica* community in Yulin (0.66) was markedly less than that in the overgrown sand dune *A. ordosica* + *A. frigida* community in Alxa (10.61). This very high ratio was because the soil CaCO_3 increased significantly while AP decreased significantly. Tables 1 and 2

showed that, among the 4 sample plots, soil CaCO_3/AP ratios in the fixed sand dune in Yulin were lower but the coefficients were greater, which resulted from the greater variation of soil CaCO_3 content in this community.

Further analysis indicated that, compared with the semi-fixed sand dune *A. ordosica* community in Yulin, the higher soil CaCO_3/AP ratio in the fixed sand dune *A. ordosica* community in Yulin was because the increase in CaCO_3 was greater than the increase in available phosphorus.

Figure 1 Spatial distribution of soil CaCO_3/AP ratios in fixed dune of *A. ordosica* community (Yulin) and in overgrown sand dune of *A. ordosica* + *A. frigida* community (Alxa). (a) Sample plot of *A. ordosica* community in Yulin; (b) sample plot of *A. ordosica* + *A. frigida* community in Alxa.

Table 1 Statistics of soil CaCO_3/AP ratios in fixed sand dune sample plot of *A. ordosica* community in Shapotou and semi-fixed sand dune sample plot of *A. ordosica* community in Yulin

Index	Site	Num	Mean	Min	Max	SE	SD	Kurt	Skew	CV(%)
CaCO_3 content (g kg^{-1})	Shapotou	50	4.91	1.36	10.01	0.30	2.10	0.27	0.69	42.77
	Yulin	50	2.16	0.64	9.02	0.18	1.26	17.41	3.42	58.33
AP content (mg kg^{-1})	Shapotou	50	4.78	2.78	7.03	0.14	0.97	-0.11	0.42	20.29
	Yulin	50	5.01	3.74	6.86	0.11	0.78	-0.61	0.43	15.57
CaCO_3/AP ratios	Shapotou	50	1.08	0.27	2.08	0.07	0.51	-0.69	0.52	47.22
	Yulin	50	0.43	0.16	1.47	0.03	0.23	7.09	2.22	53.49

Table 2 Statistics of soil CaCO_3/AP ratios in fixed sand dune sample plot of *A. ordosica* community in Yulin and overgrown sand dune sample plot of *A. ordosica* + *A. frigida* community in Alxa

Index	Site	Num	Mean	Min	Max	SE	SD	Kurt	Skew	CV(%)
CaCO_3 content (g kg^{-1})	Alxa	100	18.89	9.10	54.81	0.66	6.61	9.38	2.43	34.99
	Yulin	100	3.55	0.21	19.13	0.39	3.86	3.66	2.01	108.73
AP content (mg kg^{-1})	Alxa	100	1.96	0.60	3.72	0.07	0.71	-0.41	0.35	36.22
	Yulin	100	6.22	1.45	12.56	0.23	2.26	-0.19	0.21	36.33
CaCO_3/AP ratios	Alxa	100	10.61	3.84	32.16	0.53	5.27	4.24	1.93	49.67
	Yulin	100	0.66	0.03	3.77	0.08	0.76	5.61	2.31	115.15

Meanwhile, compared with the fixed sand dune *A. ordosica* community in Shapotou, the higher soil CaCO_3/AP ratio in the overgrown sand dune in Alxa was because the CaCO_3 content increased while the AP decreased.

Because there were different reasons for the higher CaCO_3/AP ratios, the effect of the soil CaCO_3/AP ratio on plant density in each plant community might be different. This is discussed in detail below.

2.2 Effect of soil stoichiometry of CaCO_3/AP ratio on plant density in 4 *A. ordosica* community sample plots

Regression analysis indicated that, with the increase in soil CaCO_3/AP ratios, the density of *A. ordosica* in the community in the semi-fixed sand dune in Yulin increased logarithmically and significantly ($P=0.016$; Figure 2(a)). However, the density of *A. ordosica* decreased linearly and significantly in the community in the fixed sand dune in Shapotou with increasing CaCO_3/AP ratios ($P=0.025$; Figure 2(b)). As shown in Figure 2, the soil CaCO_3/AP ratios in both sample plots were less than 2.5, ranging from 0.3 to 0.6 in the semi-fixed sand dune in Yulin and from 0.3 to 1.8 in the fixed sand dune in Shapotou. This indicated that the effects of the soil CaCO_3/AP ratio on *A. ordosica* density differed among different habitats, even though the amplitudes of the CaCO_3/AP ratios were similar.

Figure 2 Relationship between *A. ordosica* density and soil CaCO_3/AP ratio in one-dimensional sample plots of *A. ordosica* communities in Yulin and Shapotou. (a) One-dimensional sample plot of *A. ordosica* community in Yulin; (b) one-dimensional sample plot of *A. ordosica* community in Shapotou.

Regression analysis indicated that as the soil CaCO_3/AP ratio increased, the density of *A. ordosica* in the fixed sand dune community of *A. ordosica* in Yulin increased significantly and logarithmically ($P=0.000$, Figure 3). In the overgrown sand dune community of *A. ordosica* + *A. frigida* in Alxa, the density of *A. ordosica* also increased significantly and logarithmically ($P=0.002$; Figure 4(b)) while the density of *A. frigida* decreased significantly and logarithmically ($P=0.002$; Figure 4(b)). In the fixed sand dune in Yulin, the soil CaCO_3/AP ratios ranged from 0.1 to 1.5; but in the overgrown sand dune in Alxa, the soil CaCO_3/AP ratios ranged from 5 to 15. This indicated that in different habitats, though the amplitude of soil CaCO_3/AP ratios was different, the effect of CaCO_3/AP ratio on *A. ordosica* density may be

Figure 3 Relationship between *A. ordosica* density and soil CaCO_3/AP ratios in two-dimensional sample plot of *A. ordosica* community in Yulin.

Figure 4 Relationships between the densities of *A. ordosica* and *A. frigida* and soil CaCO_3/AP ratios in two-dimensional sample plot of *A. ordosica* + *A. frigida* community in Alxa. (a) *A. ordosica*; (b) *A. frigida*.

the same; however, in the same habitat, similar CaCO_3/AP ratios might have different effects on the density of different species.

2.3 Orthogonal test on pot-grown *A. ordosica* seedlings

We conducted a pot experiment with an orthogonal design using *A. ordosica* seedlings and different ratios of CaCO_3 and available phosphorus. As shown in Figure 5, soil AP had the greatest impact on the aboveground biomass of *A. ordosica*, while the interaction of soil CaCO_3 and AP ($\text{CaCO}_3 \times \text{AP}$ in Figure 5, i.e., soil CaCO_3/AP ratio) also had greater impact. However, the soil stoichiometry of the CaCO_3/AP ratio had the greatest impact on root weight of *A. ordosica* seedlings, while soil CaCO_3 and AP did not have significant effects as single factors.

3 Discussion

The soil stoichiometry of the CaCO_3/AP ratio can affect plant density. This may be an effect of variations in biological stoichiometry that result from changes in soil stoichiometry. The types and amplitudes of increases in soil stoichiometry are also important.

Our investigations in 2004 indicated that increasing soil CaCO_3/AP ratios resulted in increased *A. ordosica* density in the semi-fixed sand dune *A. ordosica* community in Yulin, but decreased density in the fixed sand dune *A. ordosica* community in Shapotou. This observation indicated that the soil stoichiometry of the CaCO_3/AP ratio was a significant factor influencing changes in plant density. The change in *A. ordosica* density in the fixed sand dune population in Shapotou was consistent with previous research, while the increase in density in the semi-fixed sand dune population in Yulin was somewhat confusing. The sample plot in Shapotou is located in the Tengger Desert, an arid area. With fixation of the dune, active microorganisms in the soil promoted decomposition of litter of *A. ordosica*, accelerating

the release of Ca^{2+} ions [19]. Therefore, in the Shapotou area, the total Ca^{2+} content in the soil was relatively high, resulting in a large reservoir of exchangeable calcium [13] that directly leads to the formation of the insoluble Ca-P phase [10,20], e.g., dicalcium phosphate and octacalcium phosphate [11,21]. On the other hand, with fixation of the dune, the soil CaCO_3 content in Shapotou increased significantly [17], which increased soil pH and precipitated Ca^{2+} and phosphorus [10,22], decreasing availability of soil phosphorus [23]. Thus, the soil stoichiometry of the CaCO_3/AP ratio was negatively correlated with *A. ordosica* density in Shapotou. However, in the semi-fixed sand dune of Yulin, the soil CaCO_3/AP ratio was positively correlated with *A. ordosica* density; that is, the increase in the soil CaCO_3/AP ratio and the increase in soil CaCO_3 content meant that the limitation of soil CaCO_3 on phosphorus was not so severe. As noted above, the semi-fixed sand dune of *A. ordosica* in Yulin is located in Mu Us Sand Land, a semi-arid area. The average annual precipitation in the Yulin sample plot (414 mm) was greater than that in the Shapotou sample plot (186.6 mm), the average soil CaCO_3 content in the Yulin sample plot (2.16 g kg^{-1}) was lower than that in the Shapotou sample plot (4.91 g kg^{-1}), while the average AP content in Yulin (5.01 mg kg^{-1}) was greater than that in the Shapotou sample plot (4.78 mg kg^{-1}). Therefore, the soil CaCO_3 only slightly limited the available phosphorus. Meanwhile, the relatively lower level of soil CaCO_3 meant that *A. ordosica* was subjected to calcium-limited conditions. Because of the importance of Ca^{2+} ions for drought-tolerance of plants [24–26], under calcium-limited conditions, an increase in Ca^{2+} ions can favor plant growth. Under calcium-deficient conditions, various processes in the cell compete for calcium [27]. In the semi-fixed sand dune sample plot in Yulin, Ca^{2+} ions dissolved out from soil CaCO_3 and there was relatively high phosphorus availability, both of which were advantageous for plant growth. Thus, the soil stoichiometry of CaCO_3/AP ratios was positively correlated with *A. ordosica* density in the semi-fixed sand dune in Yulin.

Next, we examined whether the relationship between the soil CaCO_3/AP ratio and *A. ordosica* density was the same for the fixed sand dune *A. ordosica* community in Yulin. In 2009, we also investigated the *A. ordosica* community in the fixed sand dune system in Yulin. Our results indicated that the density of *A. ordosica* increased significantly with increasing soil CaCO_3/AP ratios. Therefore, the relationship between the soil CaCO_3/AP ratio and the density of *A. ordosica* was the same in *A. ordosica* communities in both the fixed and semi-fixed sand dune systems in Yulin. As discussed in the results section, the fixed dune of *A. ordosica* community in Yulin showed slightly higher soil CaCO_3 , available phosphorus, and soil CaCO_3/AP ratios compared with the semi-fixed sand dune community, but the amplitudes were similar. Thus, *A. ordosica* plants were growing in calcium-limited conditions in the fixed dune plot in Yulin,

Figure 5 Results of three-factor orthogonal design pot experiment using *A. ordosica* seedlings.

and hence, the soil CaCO_3/AP ratio was still positively correlated with *A. ordosica* density.

However, in 2009, we investigated the overgrown dune plot of the *A. ordosica* + *A. frigida* community in the Tengger Desert, Alxa, and obtained completely different results. In the present study, the density of *A. ordosica* increased with increasing soil CaCO_3/AP ratios in the overgrown sand dune sample plot of the *A. ordosica* + *A. frigida* community in Alxa. The results of statistical analyses showed that the average soil CaCO_3 content in the sample plot in Alxa (18.89 g kg^{-1}) was far greater than that in the sample plot in Shapotou, while the average soil AP content (1.96 g kg^{-1}) was far less than that in the sample plot of Shapotou. This resulted in a far greater average soil CaCO_3/AP ratio in Alxa (10.61) than in Shapotou (1.08). Therefore, there were marked differences in the amplitudes of soil CaCO_3 content, AP content, and soil CaCO_3/AP ratios between the plots in Alxa and Shapotou. Thus, we inferred that the increased restriction of soil CaCO_3 on phosphorus availability created severely limited phosphorus conditions for growth of plants in the overgrown dune plot of the *A. ordosica* + *A. frigida* community in Alxa. This resulted from the significant increase in soil CaCO_3 and the significant decrease of available phosphorus. As discussed above for the sample plot in Shapotou, the increase of soil CaCO_3 content in an arid area would restrict phosphorus availability, resulting in the decrease in *A. ordosica* density with increasing soil CaCO_3/AP ratios in Shapotou. However, in the Alxa sample plot, *A. ordosica* density increased with increasing soil CaCO_3/AP ratios. What is the reason for this discrepancy? We speculate that the difference was because of the significant increase in soil CaCO_3 content and the significant decrease in AP content. In the sample plot in Alxa, the total biomass of the plant community remained constant over a long period because of the long-term fixation of the dune (almost 60 years). Therefore, most of the phosphorus released by organic matter/leaf litter was taken up by plants, leading to a significant decrease of available phosphorus in the soil. However, the soil CaCO_3 content increased with increasing organic matter [28] because little soil CaCO_3 was used by plants. In this case, therefore, the effects of the high soil CaCO_3/AP ratios, which reflected high soil CaCO_3 content but low AP content, on the density of *A. ordosica* actually reflected phosphorus limitation. That is to say, the soil CaCO_3 more strongly restricted phosphorus availability, and an increase in the soil CaCO_3/AP ratio resulted in a decrease in available phosphorus. In the present study, in the sample plot in Alxa, the increase in the density of *A. ordosica* with increasing soil CaCO_3/AP ratio was related to the decrease in soil available phosphorus. In other words, the density of *A. ordosica* decreased with increasing soil AP in the plot in Alxa. In fact, regression analysis indicated that the density of *A. ordosica* decreased with increasing soil available phosphorus in the overgrown sand dune of Alxa (regression equation: $y = 0.1375\ln(x) + 0.3607$, $R^2 = 0.0552$, $P = 0.019$).

Therefore, in the sample plot in Alxa, when soil CaCO_3 content increased alongside a decrease in soil, the density of *A. ordosica* increased with increasing soil CaCO_3/AP ratios.

It is worth pointing out that in the overgrown sand dune *A. ordosica* + *A. frigida* community in Alxa, the plant community is in succession, i.e., *A. frigida* is replacing *A. ordosica*. In the present study, increasing soil CaCO_3/AP ratios were associated with increased density of *A. ordosica* and decreased density of *A. frigida*, which indicated that changes in soil CaCO_3/AP ratios can affect succession of plant communities. This is not only because differences in reproductive strategies between different species in the *Artemisia* genus [29], or the influence of water on plant growth in arid regions [30,31], but also because of the impact of the soil CaCO_3/AP ratio, and especially soil available phosphorus. As mentioned above, *A. ordosica* density decreased with the increase of soil AP in the overgrown sand dune in Alxa. On the other hand, in this plant community, *A. frigida* density increased with increasing soil AP (regression equation: $y = -0.1985\ln(x) + 0.0943$, $R^2 = 0.1703$, $P = 0.000$). There is a strong relationship between phosphorus use efficiency and plant drought resistance [32]; therefore, *A. frigida* has greater phosphorus-use efficiency than *A. ordosica*. Moreover, there are differences in root mortality and root exudation between *A. ordosica* and *A. frigida* [21]. All of these differences resulted in the increase in the density of *A. ordosica* and the decrease in the density of *A. frigida* with increasing soil CaCO_3/AP ratios. On the other hand, the average soil CaCO_3/AP ratio in the sample plot in Alxa was 10.61. Thus, we could infer that in the desert-steppe of this arid region [33], when the soil stoichiometry of the CaCO_3/AP ratio exceeds 10, plant succession within the community may occur as the soil CaCO_3/AP ratio increases.

From previous research, it is clear that both soil CaCO_3 [34,35] and AP [23,36] are important for plant growth. In this study, we have shown that the soil CaCO_3/AP ratio also influences the density of *A. ordosica*. Which factor among CaCO_3 , available phosphorus, and the CaCO_3/AP ratio in soil has the greatest impact on plant growth? To address this question, in 2010 we performed a pot experiment with a three-factor orthogonal design using *A. ordosica* seedlings. The experimental conditions were based on the local status of soil CaCO_3 and AP as well as precipitation in the natural distribution area of *A. ordosica*. The results indicated that the soil CaCO_3/AP ratio had the greatest influence on aboveground biomass and root weight of *A. ordosica* seedlings. This indicated that soil CaCO_3/AP ratio had an important impact on the *A. ordosica* biomass. The influence of soil CaCO_3/AP ratio on plant biomass would ultimately lead to a change in plant density, which is consistent with our results above.

However, as discussed above, the influence of soil stoichiometry of the CaCO_3/AP ratio on *A. ordosica* density was related to the climatic zone and the types of increases in the CaCO_3/AP ratio. In the arid region, when the soil

CaCO_3/AP ratio was less than 2.5, the density of *A. ordosica* increased as the soil CaCO_3/AP ratio increased. When the soil CaCO_3/AP ratio was within the same amplitude, i.e., below 5, even though the increase in the CaCO_3 content was greater than that of soil available phosphorus, the *A. ordosica* density increased with increasing soil CaCO_3/AP ratios. This result indicated that the relationship between the soil CaCO_3/AP ratio and the *A. ordosica* density remained the same. However, in the arid region, although the soil CaCO_3/AP ratio was within the same amplitude, i.e., less than 2.5, the density of *A. ordosica* decreased with increasing soil CaCO_3/AP ratios, which was very different from the case in the semi-arid region. When the amplitude of soil CaCO_3/AP ratio was greater, that is, ranging from 5 to 15 (average, 10.61), the soil CaCO_3 content increased significantly while the soil AP decreased significantly. In this case, the density of *A. ordosica* increased with increasing soil CaCO_3/AP ratios, which meant that the relationship between the soil CaCO_3/AP ratio and the density of *A. ordosica* changed. Thus, if the reasons for the increase in the CaCO_3/AP ratio differ, the effect of soil stoichiometry of the CaCO_3/AP ratio on plant density will change.

- 1 Elser J J, Fagan W F, Denno R F, et al. Nutritional constraints in terrestrial and fresh water food webs. *Nature*, 2000, 408: 578–580
- 2 Wang S Q, Yu G R. Ecological stoichiometry characteristics of ecosystem carbon, nitrogen and phosphorus elements (in Chinese). *Acta Ecol Sin*, 2008, 28: 3937–394
- 3 Wu W, He X D, Zhou Q X. Review on N:P stoichiometry in ecosystem (in Chinese). *J Desert Res*, 2010, 30: 296–302
- 4 Elser J J, Sterner R W, Gorokhova E, et al. Biological stoichiometry from genes to ecosystems. *Ecol Lett*, 2000, 3: 540–550
- 5 Koerselman W, Meuleman A F M. The vegetation N:P ratio: A new tool to detect the nature of nutrient limitation. *J Appl Ecol*, 1996, 33: 1441–1450
- 6 Venterink H O, Wassen M J, Verkoost A W M, et al. Species richness-production patterns differ between N-, P-, and K-limited wetlands. *Ecology*, 2003, 84: 2191–2199
- 7 Silberbush M, Waisel Y, Kafkafi U. The role of soil phosphorus in differentiation of edaphic ecotypes in *aeilops peregrine*. *Oecologia*, 1981, 49: 419–424
- 8 Hinsinger P. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: A review. *Plant Soil*, 2001, 237: 173–195
- 9 Duniway M C, Herrick J E, Monger H C. Spatial and temporal variability of plant-available water in calcium carbonate-cemented soils and consequences for arid ecosystem resilience. *Oecologia*, 2010, 163: 215–226
- 10 Tunesi S, Poggi V, Gessa C. Phosphate adsorption and precipitation in calcareous soils: The role of calcium ions in solution and carbonate minerals. *Nutr Cycl Agroecosyst*, 1999, 53: 219–227
- 11 Von Wandruszka R. Phosphorus retention in calcareous soils and the effect of organic matter on its mobility. *Geochem Trans*, 2006, 7: 6
- 12 Cross A F, Schlesinger W H. Biological and geochemical controls on phosphorus fractions in semiarid soils. *Biogeochem*, 2001, 52: 155–172
- 13 Braschi I, Ciavatta C, Giovannini C, et al. Combined effects of water and organic matter on phosphorus availability in calcareous soils. *Nutr Cycl Agroecosyst*, 2003, 67: 67–74
- 14 Tilman D. The resource-ratio hypothesis of plant succession. *Am Nat*, 1985, 125: 827–852
- 15 Zhu Z D. Desert in China (Recension) (in Chinese). Beijing: Science Press, 1980
- 16 Zhao X L. Discussion on sand-fixing plants in Shapotou area. In: Institute of Desert Research, Chinese Academy of Sciences, Lanzhou. Study on Controlling Shifting Sand in Tengri Desert (2nd) (in Chinese). Yinchuan: Ningxia People Press, 1991. 27–57
- 17 Wang H T, Xue P P, He X D, et al. Changes of soil substrates in *Artemisia Ordosica* succession series (in Chinese). *Acta Sci Naturalium Univ Nankaiensis*, 2007, 40: 87–91
- 18 Bao S D. Analytical Methods of Soil and Agro-chemistry (in Chinese). Beijing: Chinese Agriculture Press, 2000
- 19 Wang H T, Zhang Y T, He X D, et al. Litterfall and decomposition of biomass and soil respiration of *Artemisia Ordosica* communities in arid region (in Chinese). *J Desert Res*, 2007, 27: 455–459
- 20 Carreira J A, Vinegla B, Laytha K. Secondary CaCO_3 and precipitation of P-Ca compounds control the retention of soil P in arid ecosystems. *J Arid Environ*, 2006, 64: 460–473
- 21 Ma B, Zhou Z Y, Zhang C P, et al. Inorganic phosphorus fractions in the rhizosphere of xerophytic shrubs in the Alxa Desert. *J Arid Environ*, 2009, 73: 55–61
- 22 Zhao Q, Zeng D H, Lee D K, et al. Effects of *Pinus sylvestris* var. *mongolica* afforestation on soil phosphorus status of Keerqin Sandy Lands in China. *J Arid Environ*, 2007, 69: 569–582
- 23 Arnesen G, Beck P S A, Engelskjøn T. Soil acidity, content of carbonates and available phosphorus are the soil factors best correlated with alpine vegetation: Evidence from Trom, North Norway. *Arc Antarc Alpine Res*, 2007, 39: 189–199
- 24 Larkindale J, Knight M R. Protection against heat stress-induced oxidative damage in *Arabidopsis* involves calcium, abscisic acid, ethylene, and salicylic acid. *Plant Physiol*, 2002, 128: 682–695
- 25 Kawano T, Sahashi N, Takahashi K, et al. Salicylic acid induces extracellular superoxide generation followed by an increase in cytosolic calcium ion in tobacco suspension culture: The earliest events in salicylic acid signal transduction. *Plant Cell Physiol*, 1998, 39: 721–730
- 26 Peng H X, Sivasithamparan K, Turner D W. Chlamydo-spore germination and fusarium wilt of banana plantlets in suppressive and conductive soils are affected by physical and chemical factors. *Soil Biol Biochem*, 1999, 31: 1363–1374
- 27 Simon E W. The symptoms of calcium deficiency in plants. *New Phytol*, 1978, 80: 1–15
- 28 Zhang N, He X D, Gao Y B, et al. Pedogenic carbonate and soil dehydrogenase activity in response to soil organic matter in *Artemisia ordosica* community. *Pedosph*, 2010, 20: 229–235
- 29 Liu Z M. Plant Reproductive Strategy in Horqin Sandy Land (in Chinese). Beijing: Meteorological Press, 2010
- 30 Zhao W Z, Cheng G D. Comments on several questions of ecological hydrological process in arid region (in Chinese). *Chin Sci Bull*, 2001, 46: 1851–1857
- 31 Yan J F, Chen X, Luo G P, et al. Response of spatial variability of underground water level on land-use and land-cover change in oasis in arid regions (in Chinese). *Chin Sci Bull*, 2006 (Chin Ver), 51(Suppl I): 42–48
- 32 Gnanasire S. Cell membrane stability and leaf water relation as affected by phosphorus nutrition under stress in Maize. *Soil Sci Plant Nutr*, 1990, 36: 661–666
- 33 Liu Y X. Phyto-reclamation of sand dunes in northwest, north and northeast of China (in Chinese). *J Desert Res*, 1988, 8: 11–17
- 34 Zuo Y, Ren L, Zhang F, et al. Bicarbonate concentration as affected by soil water content controls iron nutrition of peanut plant in a calcareous soils. *Plant Physiol Biochem*, 2007, 45: 357–364
- 35 Pedersen J, Fransson A M, Olsson P A. Performance of *Anisantha (Bromus) tectorum* and *Rumex acetosella* in sandy calcareous soil. *Flora*, 2011, 206: 276–281
- 36 Holford I C R. Soil phosphorus: Its measurement, and its uptake by plants. *Aus J Soil Res*, 1997, 35: 227–240