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The optimal Redfield N: P ratio caused by fairy ring fungi stimulates plant productivity in the temperate steppe of China

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ABSTRACT

The Redfield ratio (or stoichiometry) is widely used as a reference to differentiate between nitrogen (N) and phosphorus (P) limitations in plankton and in the deep oceanic waters. Whether the Redfield ratio is applicable to plant and soil fungal growth is rarely considered. Fairy rings (FRs) caused by *Agaricus gennadii* can influence plant productivity and soil nutrient composition. However, the internal mechanisms associated with the optimal Redfield N: P ratio remain unclear. Changes in plant and soil stoichiometry in three concentric zones of FRs occurring in the temperate steppe of China, namely outside the ring (Out), on the ring (On), and inside the ring (In), were assessed in order to confirm the effects of FR fungi on plants and soil and explain the mechanisms using nutrient stoichiometry. We observed a significantly higher aboveground biomass only in the On zone. The plant N: P ratio was 14.5, 10.4, and 7.3 in the Out, On, and In zones, respectively. This indicates that the limiting element of the aboveground biomass was P in the Out zone and N in the In zone, while the On zone possessed an optimal plant N: P ratio and relatively high aboveground biomass. As with the plant N: P ratio, the On and In zones displayed a significantly lower soil inorganic N: available P ratio than the Out zone. The plant N: P ratio was highly positively correlated with the soil inorganic N: available P ratio. Our results suggest that FR fungi with low N: P ratio shift the soil ecosystem from P- to N-limited, thereby accelerating the uptake of soil N by plants. As with the plant N: P ratio, the soil inorganic N: available P ratio can also be used to evaluate whether N or P is more limiting for plant production in grassland ecosystems.

1. Introduction

Nitrogen (N) and phosphorus (P) are the two most limiting elements in terrestrial plant growth (Reich and Oleksyn, 2004; Elser et al., 2007). Increases in N deposition have pervasive impacts on nutrient cycling (Aber et al., 2003), which may cause terrestrial ecosystems to transition from N- to P-limited (Menge and Field, 2007; Braun et al., 2010; Penuelas et al., 2013; Deng et al., 2016). Redfield was first to propose that the ratio of N to P in plankton might be the cause of the global ocean's remarkably similar ratio of dissolved N: P, 16:1 (Redfield, 1958). Klausmeier et al. (2004) predicted that the canonical Redfield N: P ratio of 16 is not a universal biochemical optimum, but instead represents an average of species-specific N: P ratios. Cleveland and Liptzin (2007) reported that the Redfield N: P ratio in both the soil (13: 1) and the soil microbial biomass (7: 1) are well-constrained at the global scale. More recently, Loladze and Elser (2011) suggested that Redfield N: P ratio relates to a more fundamental homeostatic balance between protein and rRNA. This ratio in the leaves of plants also has been used to evaluate whether N or P is more limiting for plant biomass (Gusewell, 2004). The relative concentrations of N and P in plant leaves in wetlands (Koerselman and Meuleman, 1996; Gusewell et al., 2003; Gusewell, 2005), forests (Townsend et al., 2007; Veresoglou et al., 2014), and grasslands (Han et al., 2005; Craine et al., 2008; He et al., 2008; Craine and Jackson, 2010) have been extensively studied. Generally, an N: P ratio <14 indicates N limitation, while a ratio >16 suggests P limitation (Koerselman and Meuleman, 1996). Previous studies have overwhelmingly improved our understanding of the variations in N and P in plants. Similarly, the ratio of soil N and P may also be used as a discriminating value (Bui and Henderson, 2013), and this ratio in particular

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is influenced by artificial fertilization (Penuelas et al., 2012). However, most studies on the N: P ratio have focused on leaves, and the relationship of this ratio between plants and soil across ecosystems remains unclear, particularly in grasslands where the availability of soil nutrients is a critical determinant of ecological dynamics (Craine and Jackson, 2010). It is therefore necessary to assess plant nutrient limitation under disturbed natural conditions in order to obtain an accurate reflection of the conditions of the study area.

Fairy rings (FRs) are caused by basidiomycete fungi, which grow radially through the soil and produce fruit bodies near the outer edge of the ring (Edwards, 1984). More than 50 species of basidiomycete, which grow below-ground in a regular and radial fashion, can produce FRs (Fox, 2006). They are often classified into three types according to whether the vegetation (i) is killed at the ring margin (type I), (ii) grows more vigorously (type II), or (iii) is unaffected (type III) (Shantz and Piemeisel, 1917; Griffith and Roderick, 2008). In type II, the vegetation in the fungal growth zone grows vigorously, as evidenced by the dark-green vegetation boundaries (Fig. 1). Early research has suggested that FR fungi grow on dead soil organic matter and release nutrients into the soil, resulting in higher availability of both N and P within the highly vegetated area of the ring than outside the ring (Edwards, 1988; Kaiser, 1998). Although nutrient accumulation by FR fungi has been investigated with regards to plant growth stimulation (Shantz and Piemeisel, 1917; Fisher, 1977; Edwards, 1984, 1988; Xu et al., 2011; Bonanomi et al., 2012), there have been no further studies on the underlying mechanisms. The manner in which FR fungi alter the N and P concentration of plants and soil, and hence stimulate plant productivity, remains unknown. Whether this stimulation is associated with a change in the N: P ratio of the plants and soil by FRs has not yet been elucidated.

The temperate steppe of China is mainly distributed in Inner Mongolia and constitutes a large part of the Eurasian steppe (Yang et al., 2012). The FR regions in this study are close to the south-eastern part of Inner Mongolia. This area has a semi-arid continental monsoon climate, which is dominated by warm, moist air currents from the Pacific Ocean in summer, while autumn, winter, and spring are dominated by cold, dry air currents from Mongolia (Rong et al., 2015). The basidiomycete *Agaricus gennadii* develops FRs. Plants in the fungus growth zone grow well (Fig. 1A) and provide a distinctive natural environmental setting to test: (1) how the FR fungi affect the productivity of the plants; (2) how the FR fungi affect the N: P ratio of the plants; and (3) the relationship between plant N: P ratio and soil N: P ratio. Based on this, we addressed the following hypotheses: (1) The FR fungi stimulated plant productivity by changing the N: P ratio. (2) FR fungi reduce the N: P ratio of the plants by increasing the concentration of P. (3) The plant N: P ratio is related to the ratio of available soil N: P, but not the soil total N: P ratio.

2. Materials and methods

2.1. Study sites

This study was carried out at the National Field Station for Grassland Ecosystems, which is close to the south-eastern part of Inner Mongolia, China (41°44′N, 115°40′E, elevation 1430 m). This area is a typical temperate zone characterized by a mean annual precipitation of 430 mm and a mean annual temperature of 1.4 °C. The minimum monthly mean air temperature is −18.6 °C in January and a maximum of 21.1 °C occurs in July. Precipitation mainly falls during the growing season (June to August), which coincides with the highest temperatures. The plant community is dominated by *Leymus chinensis*. The site has a calcic-orthic Aridisol soil with a loamy-sand texture according to the ISSS Working Group RB, 1998 (Yang et al., 2017).

2.2. Plant and soil sampling

Three regular FRs with an outer diameter of ~5 m were investigated. The ring-producing fungus (Fig. 1C) was identified by DNA molecular authentication as *A. gennadii* (Fig. S1). Three different zones were identified from the outer to the inner areas of the rings. These were defined as: Out = zone outside of where the fungus occurs; On = zone with flourishing vegetation; and In = internal area of the ring (Fig. 1A).

Soil cores were collected from the topsoil (15-cm depth and 5-cm diameter) from four different directions for each ring (Fig. 1B). Three different zones were collected in each direction, and each zone was at a distance of 100 cm. Three soil cores at a distance of 50 cm were combined as one sample for each direction. Then, 180 soil samples (three rings, three zones, four directions, and five months) were collected from June to October 2016. We mixed the four direction samples into a repeated sample, and obtained a

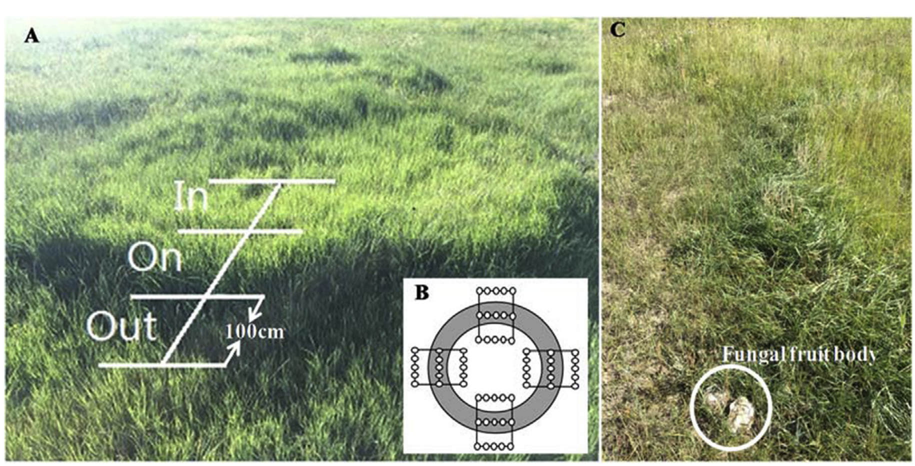

Fig. 1. *Agaricus gennadii* fungus develops a fairy ring as evidenced by the green vegetation boundary. **(A)** Indicates the three zones: (Out) outside the fairy ring, (On) on the zone with fungus growth, and (In) the inner zone. **(B)** Soil samples were collected from the topsoil (0–15 cm) from four directions and three zones. **(C)** *Agaricus gennadii* fruit bodies appeared in the edge between the Out and the On zones. Image by Chao Yang on May 26 (A) and August 28 (C), 2017 at Guyuan County (41°44′N, 115°40′E).

representative fairy ring data point. Over the study period, we took a total of 45 soil samples (three rings, three zones and five months) for incubation and analysis. We were careful to minimise damage to the areas adjacent to the sampled areas. The soil samples of the next month were collected from adjacent to the sample from the previous month in the same rings. The samples were transferred to the laboratory and sieved through a 2-mm mesh. Soil samples were dried at room temperature for chemical analyses.

To reduce the damage to the FR, one quadrat (20×20 cm) was established only in one direction to determine the aboveground biomass of the community. Then, 45 vegetation samples (three rings, three zones, one direction, and five months) were also collected from June to October 2016. The vegetation samples of the next month were collected from adjacent to the sample from the previous month in the same rings. Shoots were cut at the soil surface and oven dried at 65 °C for 72 h before being weighed. The dominant plant species was *L. chinensis*, and there were no differences in species richness between the three zones. However, the species abundance changed significantly (Table S1). We counted the number of fruit bodies in three zones in August 2016, and the fruiting bodies were also collected and oven dried at 65 °C for 72 h to determine the N and P concentrations. The results are indicated in Table S2.

2.3. Laboratory analysis

Soil was analysed for total N, total P, inorganic N (NH_4^+-N and NO_3^--N), available P, total C and pH. We analysed plant N and P concentrations in the aboveground biomass of the community, in addition, the N and P concentrations of the FR fruit body were also analysed. In brief, the soil total N and plant and fruit body N concentrations were measured using a FOSS Kjeltec 2300 Analyzer Unit (FOSS, Hillerød, Sweden). The soil inorganic N concentrations (NH_4^+-N and NO_3^--N) were extracted using 50 mL of 2 mol/L KCl on a 10 g subsample and analysed using a Flow-Solution analyzer (Flowsys, Ecotech, Germany). The plant and fruit body P concentrations were determined using a spectrophotometer with ammonium molybdate and ascorbic acid as colour reagents (Carter and Gregorich, 2008), following digestion of the plant tissue with nitric and perchloric acids. The soil total P was determined using the sodium hydroxide melting-molybdenum antimony colorimetric method. The soil available P (Olsen P) was extracted by shaking 1.5 g of dry soil for 30 min at 20 °C in 100 mL of a 42% $NaHCO_3$ solution (pH 8.5) according to Carter and Gregorich (2008). The soil total C concentration was measured using an auto-analyzer (TOC, Elementar, Germany), and the soil pH was measured with a glass electrode in a 1:2.5 soil: water suspension.

2.4. Molecular identification of *Agaricus gennadii*

The fresh fruit bodies of the FRs were collected in August 2016. The fungal genomic DNA was extracted using a DNAsecure Plant Kit (TIANGEN Biotech Co., Ltd.) following the manufacturer's instructions. PCR amplification of fungal ITS sequences was conducted in a volume of 25 μL containing $10 \times$ buffer 2.5 μL, Mg^{2+} (25 mmol L^{-1}) 2.5 μL, dNTP (25 mmol L^{-1}) 2 μL, 1.0 μL each of the ITS1 forward and ITS4 reverse ITS primers, 0.3 μL Taq DNA polymerase, 0.5 μL of DNA template, and nuclease-free water. The amplification was carried out over 35 cycles using a Mastercycler gradient thermocycler (Eppendorf Scientific Inc., Westbury, NY, USA) at 94 °C for 5 min (predegeneration), 94 °C for 30 s (denaturation), 52 °C for 30 s (annealing), and 72 °C for 1 min (extension). The DNA sequences were deposited in GenBank (accession numbers KY644142). Phylogenetic relationships were analysed using MEGA version 4.0, and the distances were calculated using the Kimura 2-parameter distance model. A phylogenetic tree was constructed using the neighbor-joining method. Each dataset was bootstrapped 1000 times (Fig. S1).

2.5. Statistical analysis

We averaged the four directions of soil data in each zone prior to statistical analysis. The effects of the three zones and five months on the 13 indexes of plant and soil were analysed using two-way ANOVA. One-way ANOVA was used to test whether each of the plant variables (aboveground biomass, plant N, plant P, aboveground biomass N content, aboveground biomass P content, and plant N: P ratio) and soil variables (soil total N, soil total P, soil inorganic N, soil available P, soil total C, and pH) were related to the three zones or to five months. We were specifically interested in the stoichiometry of the three zones. Therefore, we averaged the five month data of plant N: P ratio, soil total N: P ratio, and soil inorganic N: available P ratio, and analysed the differences in the three zones using one-way ANOVA. A quadratic curve model was used to explore the relationships between aboveground biomass and plant N: P ratio. Relationships between the N: P ratio and the concentrations of N and P were examined by linear regression analyses. All of the statistical analyses were performed using SPSS (version 19.0; IBM, Armonk, New York, USA), and the regression analyses and figures were produced using SigmaPlot (version 12.5, Systat Software, Inc., San Jose, CA, USA).

3. Results

3.1. FR fungi stimulates plant productivity and nutrient content

The 30.3 mean number of fungal fruit bodies were observed only in the On zone, and the N and P concentrations of fungal fruit body were 62.5 and 12.1 g kg^{-1}, respectively. The N: P ratio was 5.2, which is far below the Redfield ratio and indicates P limitation (Table S2).

The Zone, Month, and Zone $\times$ Month significantly affected the aboveground biomass, plant N, plant P, aboveground biomass N content, and aboveground biomass P content, with the exception of Zone $\times$ Month on aboveground biomass (Table 1). A significantly higher aboveground biomass only occurred in the On zone, especially in August (Fig. 2). The value of plant N and P decreased significantly with the progression of the months (Table 1, Fig. 3). Plant N and aboveground biomass N content increased significantly in each month in the On zone in comparison with the Out zone (Fig. 3A and B). Plant N decreased in the In zone in comparison with the Out zone, but aboveground biomass N content showed no difference between the Out and In zone from July to October (Fig. 3A and B). Significant increases in plant P and aboveground biomass P content were observed in the On and In zone (Fig. 3C and D).

3.2. Differences in plant stoichiometry

The plant N: P ratio was higher in the Out zone relative to the On and In zones, and the highest value was observed at the end of the growing season (Fig. 4A). Furthermore, the average value of the plant N: P ratio was 14.5, 10.4, and 7.3 for the Out, On, and In zone, respectively (Fig. 4B). The aboveground biomass showed a negative quadratic relationship with plant N: P ratio, and the vertex coordinates of the quadratic were 10.7 for plant N: P ratio and 611.9 g m^{-2} for aboveground biomass (Fig. 4C).

Table 1
Two-way ANOVA for the evaluation of the two primary factors (zone and month) that influence the 13 plant and soil variables. Significant relationships at $P < 0.001$ are given in bold.

Variables	Zone		Month		Zone × Month	
	F value	*P* value	*F* value	*P* value	*F* value	*P* value
Aboveground biomass (g m^{-2})	264.8	**< 0.001**	20.5	**< 0.001**	2.6	0.029
Plant N (g kg^{-1})	2582.3	**< 0.001**	5139.9	**< 0.001**	221.0	**< 0.001**
Plant P (g kg^{-1})	5174.0	**< 0.001**	4094.8	**< 0.001**	274.1	**< 0.001**
Aboveground biomass N content (g m^{-2})	437.0	**< 0.001**	32.3	**< 0.001**	14.7	**< 0.001**
Aboveground biomass P content (g m^{-2})	427.3	**< 0.001**	35.0	**< 0.001**	12.9	**< 0.001**
Plant N: P	3733.4	**< 0.001**	26.8	**< 0.001**	37.3	**< 0.001**
Soil total N (g kg^{-1})	58.1	**< 0.001**	60.6	**< 0.001**	3.3	0.008
Soil total P (g kg^{-1})	14.4	**< 0.001**	63.1	**< 0.001**	1.8	0.113
Soil total N: P	2.6	0.091	16.9	**< 0.001**	0.7	0.697
Soil inorganic N (mg kg^{-1})	39.7	**< 0.001**	101.4	**< 0.001**	20.5	**< 0.001**
Soil available P (mg kg^{-1})	14.7	**< 0.001**	15.0	**< 0.001**	0.8	0.581
Soil inorganic N: available P	32.8	**< 0.001**	15.0	**< 0.001**	8.2	**< 0.001**

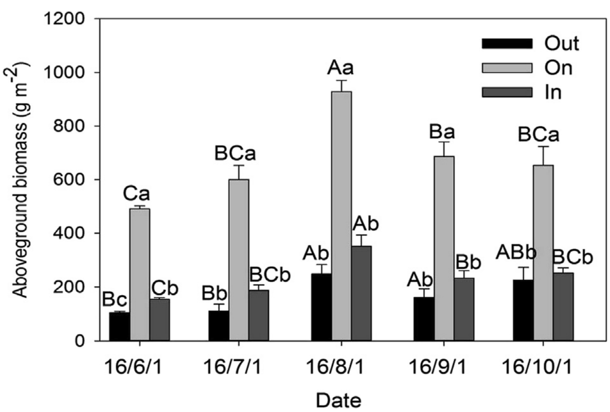

Fig. 2. Aboveground biomass each month from June 1 to October 1, 2016 in the three zones. Means with different letters are significantly different based on ANOVA and LSD ($P < 0.05$); lowercase letters indicate comparisons among the three zones and uppercase letters indicate the 5-month comparisons.

3.3. *Soil nutrient stoichiometry controls plant stoichiometry*

There were significant differences in the soil total N, total P, inorganic N, and available P between the zones or months (Table 1). Moreover, the values of these four indicators decreased significantly as the months progressed (Table 1, Fig. 5). FRs decreased the soil total N, total P, and inorganic N in the On and In zones compared to the Out zone (Fig. 5A–C), but increased available P in the soil (Fig. 5D). The soil concentrations of total C were significantly lower in the On and In zones than the Out zone. However, the soil pH value was significantly higher in the On and In zone than in the Out zones, except during September and October (Fig. S2).

As with the plant N: P ratio, the On and In zone had a significantly lower soil inorganic N: available P ratio than that of the Out zone (Fig. 6A). However, there was no significant difference in the soil total N: P ratio between the three zones (Fig. 6B). In general, the plant N: P ratio exhibited a significant linear relationship with plant P and the soil inorganic N: available P ratio (Fig. 7B, D). In the Out zone, the plant N: P ratio showed a significant linear relationship with plant N, plant P, soil total N: P ratio, and soil inorganic N: available P ratio (Fig. S3A-D). By contrast, the plant N: P ratio only showed a significant linear relationship with soil inorganic N: available P ratio in the On zone (Fig. S3H), and displayed a significant linear relationship with plant P and the soil total N: P ratio in the In zone (Fig. S3J, K). Soil total N: P ratio exhibited a negative linear relationship with soil total N, soil total P, and soil inorganic N: available P ratio (Fig. 7E–G), and those relationships were also observed in the Out and On zone (Fig. S4A-F). However, in the In zone, negative correlations were observed only between the soil total N: P ratio and soil total P (Fig. S4H). Moreover, positive correlations between the soil inorganic N: available P ratio and soil inorganic N were detected in all three zones (Fig. 7H; Fig. S5A, C, E), but soil inorganic N: available P showed a positive linear relationship with soil available P only in the On zone (Fig. S5D).

4. Discussion

FRs caused by *A. gennadii* had a clear effect on the spatial (zone) and temporal (month) dynamics of the plants and soil. Although all the soil nutrient variables decreased as the months progressed, plant growth was stimulated throughout the entire growing season in the On zone where the FR fungi was the most active in influencing soil properties (Table S2).

4.1. *FR fungi stimulates plant productivity and nutrient content*

To our knowledge, this is the first report showing the spatio-temporal dynamics of FRs and the systematic detection of the effects of FRs on plants and soil. Earlier studies reported changes in vegetation and soil nutrients by FRs (Shantz and Piemeisel, 1917; Fisher, 1977; Edwards, 1984, 1988). A previous study conducted in a permanent pasture showed that the N content of the vegetation varied significantly with its position on the ring, and was higher on the ring than outside or inside the ring (Edwards, 1988). In the present study, both the plant N and plant P were significantly higher in the On zone than in the Out and In zones. In addition, the aboveground biomass was significantly higher in the On zone than the Out and In zones, which is consistent with the results of recent studies (Bonanomi et al., 2012; Caesar-TonThat et al., 2013). When biomass and plant N and P concentration were combined, we further found that FRs promoted the transformation of N and P from the soil to the plant, resulting in the decrease of total N and P soil concentration (Edwards, 1988).

4.2. *Differences in plant stoichiometry*

In this study, aboveground biomass exhibited a negative quadratic relationship with plant N: P ratio. Previous results suggested that the N: P ratio of the foliage is negatively correlated with biomass production (Gusewell, 2004; Choi and Lee, 2015). We found an optimal value between plant N: P ratio and aboveground biomass in our study regions, namely 10.7 for plant N: P ratio and 611.9 g m^{-2} for aboveground biomass. Loladze and Elser (2011) suggested that the plant N: P ratio is often below the optimal

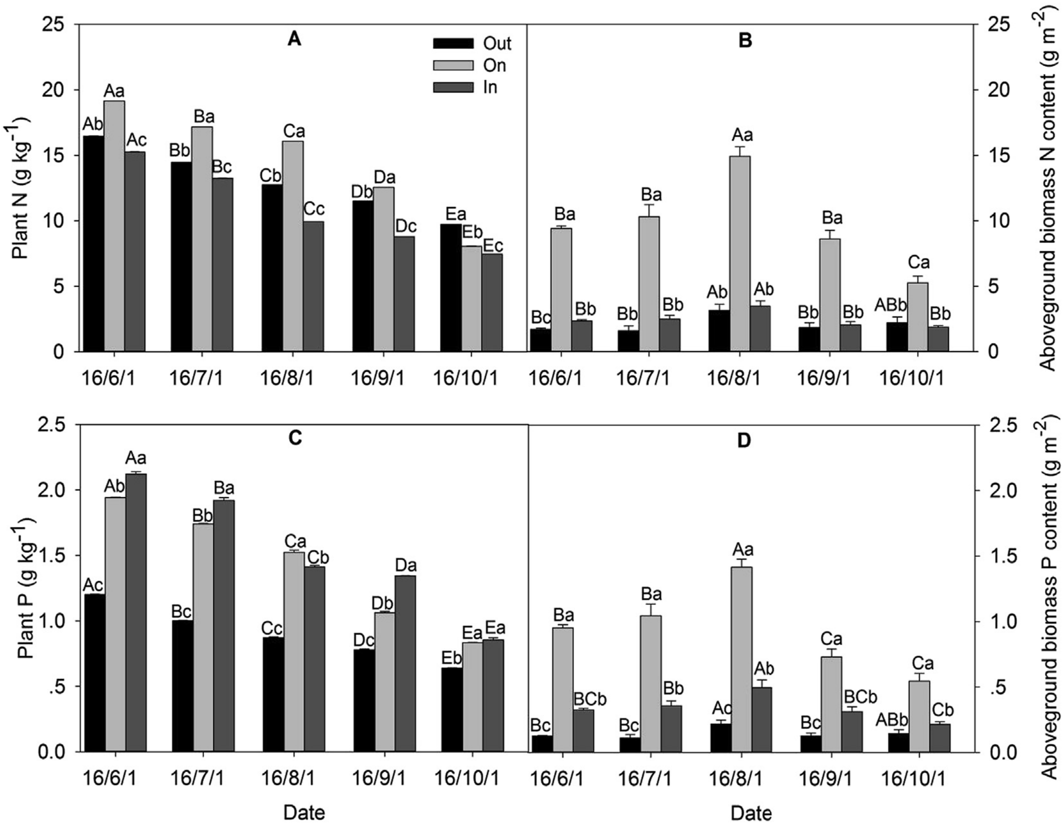

Fig. 3. Plant N **(A)**, aboveground biomass N content **(B)**, plant P **(C)** and aboveground biomass P content **(D)** each month from June 1 to October 1, 2016 in the three zones. The means with different letters are significantly different based on ANOVA and LSD ($P < 0.05$); lowercase letters indicate comparisons among the three zones and uppercase letters indicate the 5-month comparisons.

ratio in N-limited ecosystems. In contrast, the plant N: P ratio is often above the optimal ratio in P-limited ecosystems. Generally, during plant growth, there are three stages of response to continued N deposition (Aber et al., 1989; Agren, 2008; Sardans et al., 2012; Deng et al., 2016). Here we defined the three stages as nitrogen deficiency (stage one), optimal (stage two), and phosphorus deficiency (stage three). In the Out zone, we found that the plant N: P ratio was 14.5, and the aboveground biomass of the plant was almost limited by P, which corresponds to stage three according to Koerselman and Meuleman (1996). In the On zone, the plant N: P ratio decreased to 10.4 with an increase of available P in the soil by FR fungi, which is associated with stage two. Additionally, the plant N: P ratio continued to decrease to 7.3 in the In zone, indicating that the aboveground biomass of the plant had shifted from P-limited to N-limited, signifying a stage one response. Our study suggested that the limiting element of aboveground biomass of the Out zone was P, and in the In zone it was N. The On zone experienced neither P limitation nor N limitation, and was associated with relatively high aboveground biomass.

4.3. Soil nutrient stoichiometry controls plant stoichiometry

The decrease in soil total N and total P outside the fungal ring indicates major changes in N and P cycling induced by fungal activity. Similar results have been reported for FRs produced by other fungi, such as *Agaricus arvensis* (Edwards, 1984) and *Agaricus lilaceps* (Caesar-TonThat et al., 2013). Shantz and Piemeisel (1917) demonstrated that the growth of mycelium leads to a substantial breakdown of soil organic matter and hence an increase in the concentrations of ammonium nitrate in the soil. Recently, Xu et al. (2011) suggested that soil available N in the highly vegetated area is significantly higher than outside the fairy ring. The zones of stimulated growth were attributed to the enhanced concentrations of inorganic nitrogen. However, we found that the concentrations of soil available P were higher on the ring than outside the ring, and in contrast, soil inorganic N decreased from the Out zone to the On zone. There is evidence that may indirectly support this result. Total soil N and P was absorbed by the FR fungi, resulting in the high concentration of N and P in the fruit body (the N: P ratio of the fruiting body was 5.2; Table S2). When the fruit body of the fungi completes its life cycle, more P is returned to the soil than N, and the vegetation requires large amounts of N from the soil under sufficient P conditions.

The plant N: P ratio is thought by some to be indicative of nutrient limitation at the plant community level (Gusewell, 2004). By analogy, Bui and Henderson (2013) suggested that the soil N: P ratio is also of potential diagnostic value. Fan et al. (2015) found that the N: P ratio is strongly associated with plants and soil, indicating a close connection between the plant and soil nutrients. Our result showed that plant N: P ratio was significantly related to the soil inorganic N: available P ratio. This may indicate that soil nutrient availability (inorganic N: available P) can also indirectly assess whether N or P is more limiting for plant productivity. In addition, the leaf N: P ratio is significantly negatively correlated with leaf P, but not with leaf N, and the variation in the N: P ratio is primarily determined by leaf P (Gusewell, 2004; He et al., 2008), which is consistent with our findings. However, the soil inorganic N: available P ratio was highly positively correlated with soil inorganic N, but not with soil available P. One potential explanation is that the average value of the plant N: P ratio was 14.5, 10.4, and 7.3 for the

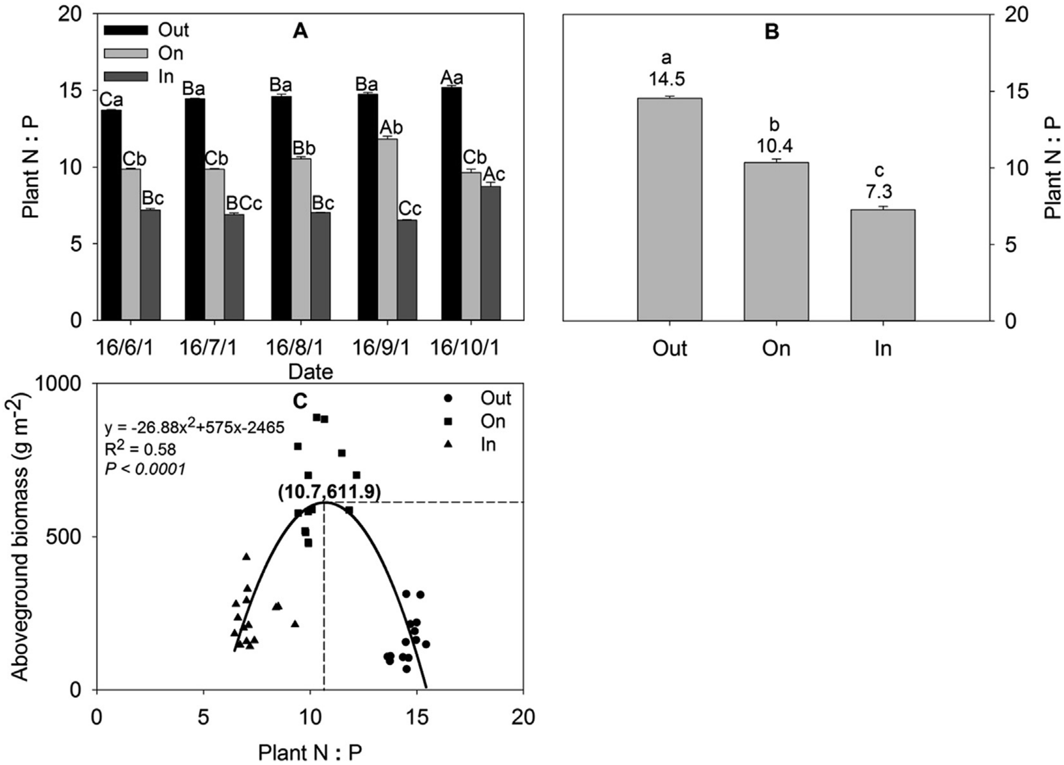

Fig. 4. Plant N: P ratio each month from June 1 to October 1, 2016 **(A)**, Plant N: P of the three zones **(B)** and the relationship between aboveground biomass and plant N: P **(C)**. The means with different letters are significantly different based on ANOVA and LSD ($P < 0.05$); lowercase letters indicate comparisons among the three zones and uppercase letters indicate 5-month comparisons.

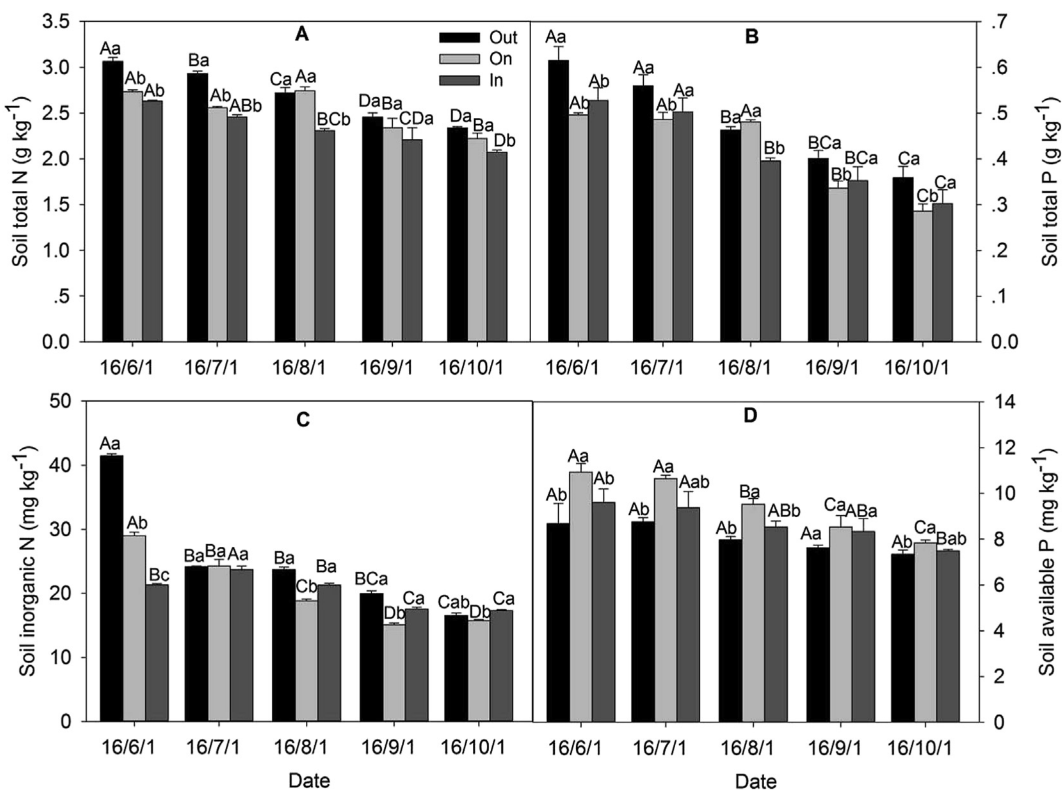

Fig. 5. Soil total N **(A)**, soil total P **(B)**, soil inorganic N **(C)**, and soil available P **(D)** each month from June 1 to October 1, 2016 in the three zones. The means with different letters are significantly different based on ANOVA and LSD ($P < 0.05$); lowercase letters indicate comparisons among the three zones and uppercase letters indicate 5-month comparisons.

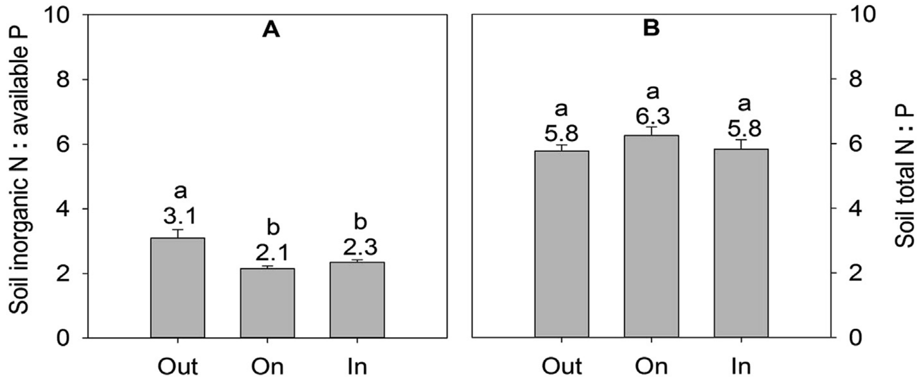

Fig. 6. Soil inorganic N: available P **(A)** and soil total N: P **(B)** of the three zones. Values are the means ± SE of 5 months. The means with different letters are significantly different based on ANOVA and LSD (*P < 0.05*).

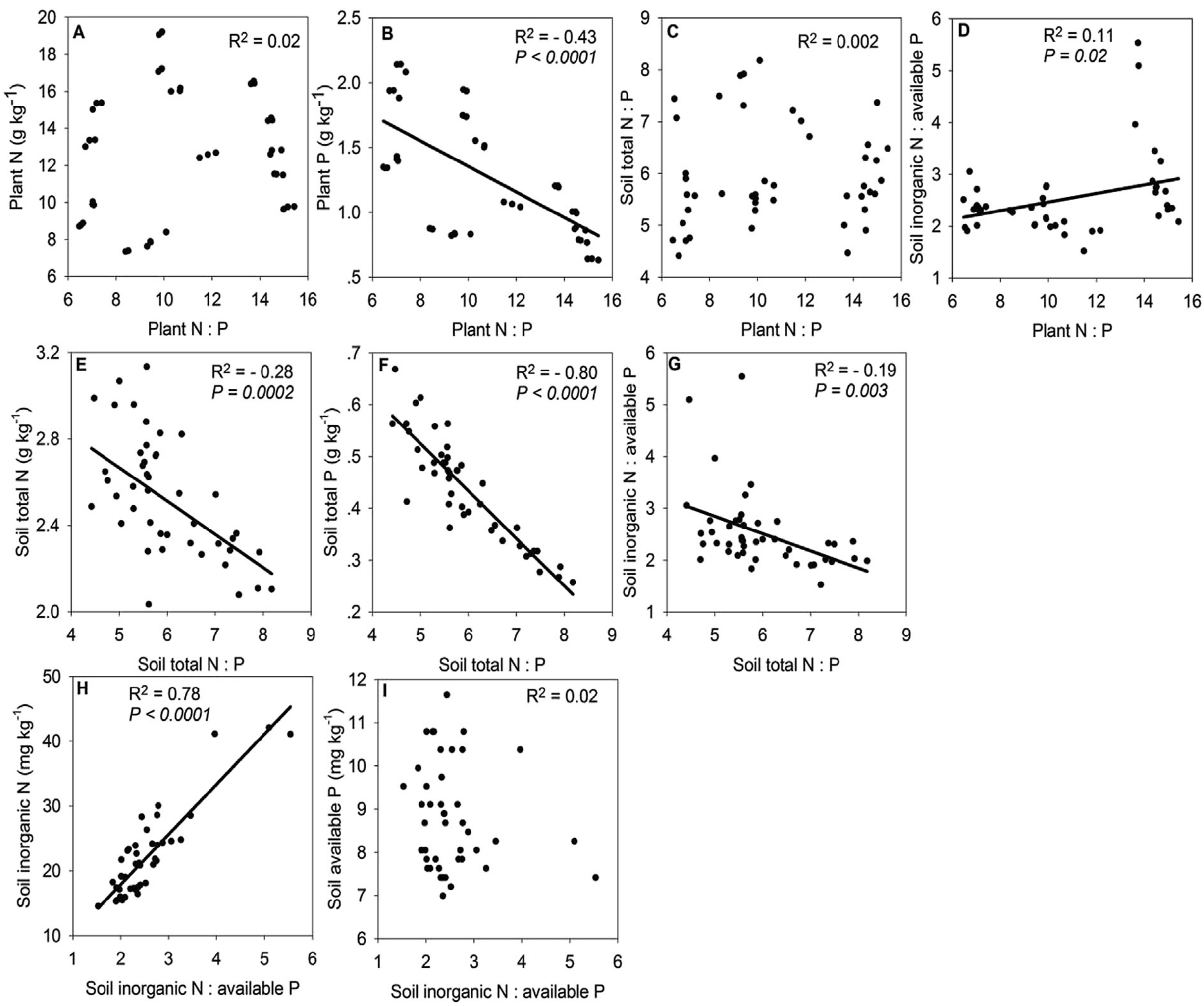

Fig. 7. Relationships between plant N, plant P, soil total N: P, soil inorganic N: available P and plant N: P **(A–D)**, soil total N, soil total P, soil inorganic N: available P and soil total N: P **(E–G)** and soil inorganic N, soil available P and soil inorganic N: available P **(H–I)**.

Out, On, and In zone, respectively, indicating that the plants in our study were almost P-limited, as according to Koerselman and Meuleman (1996). FR fungi increased the available P in soil, therefore, the plants changed from P-limited to N-limited, and thus soil inorganic N was continuously utilized by the plants. In addition, the soil available P was sufficient, and as a result, low soil inorganic N was associated with a low soil inorganic N: available P ratio. There was no statistically significant variation in the soil total N: P ratio between the three zones. Conversely, Fan et al. (2015) reported that the N: P ratio of plants increases linearly with the soil total N: P ratio. There is evidence that may indirectly explain these differences. The research area in our study was a natural ecosystem without human disturbance and fertilization, while Fan et al. (2015) fertilized the study site, thereby altering the total soil nutrient content. In the future, we suggest using the ^{15}N isotope analysis as a way of elucidating further what is happening from soil to FR fungi and plant.

5. Conclusions

The present study provides evidence that fungal FRs alter the Redfield N: P ratio of plants and soil, thereby promoting changes in the vegetation productivity of semi-arid grasslands. Here, we found that the aboveground biomass was 169.7, 671.8, and 235.1 g m^{-2} for the Out, On, and In zones, respectively. Accordingly, the plant N: P ratio was 14.5, 10.4, and 7.3, which indicates that the fungi of the FRs shifted the soil ecosystem from P- to N-limited, accelerating the uptake of soil N by plants. Our results corroborate previous findings that the variation in plant N: P ratio is primarily controlled by plant P. In addition, our results also show that the plant N: P ratio is highly positively correlated with soil inorganic N: available P, and that the soil inorganic N: available P ratio can also be used to assess whether N or P is more limiting for biomass production.

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Supplementary data

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