Plant Nitrogen Dynamics and Nitrogen-use Strategies under Altered Nitrogen Seasonality and Competition

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• Background and Aims Numerous studies have examined the effects of climatic factors on the distribution of C3 and C4 grasses in various regions throughout the world, but the role of seasonal fluctuations in temperature, precipitation and soil N availability in regulating growth and competition of these two functional types is still not well understood. This report is about the effects of seasonality of soil N availability and competition on plant N dynamics and N-use strategies of one C3 (Leymus chinensis) and one C4 (Chloris virgata) grass species.

• Methods Leymus chinensis and C. virgata, two grass species native to the temperate steppe in northern China, were planted in a monoculture and a mixture under three different N seasonal availabilities: an average model (AM) with N evenly distributed over the growing season; a one-peak model (OM) with more N in summer than in spring and autumn; and a two-peak model (TM) with more N in spring and autumn than in summer.

• Key Results The results showed that the altered N seasonality changed plant N concentration, with the highest value of L. chinensis under the OM treatment and C. virgata under the TM treatment, respectively. N seasonality also affected plant N content, N productivity and N-resorption efficiency and proficiency in both the C3 and C4 species. Interspecific competition influenced N-use and resorption efficiency in both the C3 and C4 species, with higher N-use and resorption efficiency in the mixture than in monoculture. The C4 grass had higher N-use efficiency than the C3 grass due to its higher N productivity, irrespective of the N treatment or competition.

• Conclusions The observations suggest that N-use strategies in the C3 and C4 species used in the study were closely related to seasonal dynamics of N supply and competition. N seasonality might be involved in the growth and temporal niche separation between C3 and C4 species observed in the natural ecosystems.

Key words: Competition, C3 and C4 grasses, nitrogen seasonality, nitrogen productivity, mean residence time, temperate steppe.

INTRODUCTION

Temporal niche separation of C3 and C4 plant species was primarily attributed to the seasonal temperature regime in the early 1980s (Kemp and Williams, 1980). Since then, this conclusion has seldom been challenged. Under natural conditions, however, there are concomitant temporal fluctuations in temperature, precipitation/soil water availability and soil N mineralization and availability. Given the well-documented differences in water and N-use efficiency (NUE) between C3 and C4 plants, seasonal fluctuations in soil water and/or N availability are expected to affect growth and temporal niche separation of these two plant functional types. More recently, seasonal water availability has been documented to influence both the distribution (Winslow et al., 2003) and the relative abundance (Murphy and Bowman, 2007) of C3 and C4 species. The effects of seasonal water variability on photosynthetic characteristics of C3 and C4 grasses were also addressed (Niu et al., 2005). Through altering soil N mineralization rates, seasonal fluctuation of temperature and soil water supply can cause nitrogen (N) pulses (Burke, 1989; Gallardo and Schlesinger, 1995). Because plant N uptake, transport and use are closely related to soil N availability (Lambers et al., 1998; Aerts and Chapin, 2000), N pulse can be important for the annual N supply of plants (Campbell and Grime, 1989; Robinson, 1994; Marnolos, 2006). Therefore, changes in soil N availability, including seasonal variations, might impose an impact on plant N-use strategies and potentially shift the interspecific competition between C3 and C4 species (Wedin and Tilman, 1993; Ripley et al., 2007), which could contribute to their temporal niche separation. However, no study, as far as is known, has addressed the effects of N seasonality on plant N uptake and utilization.

The temporal niche separation of C3 and C4 species has been proposed as an adaptive mechanism allowing plants to alleviate interspecific competition for limiting resources in a given habitat (Collatz et al., 1998; Epstein et al., 1999; Nelson et al., 2004). The competitiveness of a species is closely related to its particular habitat (Tilman, 1988) and with the other co-occurring species in the community. As N supply fluctuates, one can expect that competitive interactions among species also change. Altered soil N availability in an ecosystem can shift the competitive balance of C3 to C4 plants by changing the relative importance of below-ground and above-ground competition (Tilman, 1982, 1988). In tall-grass prairie (McKane et al., 1990) and short-grass steppe ecosystems (Epstein et al., 1998), differences in timing of growth between C3 and C4...
plants has led to temporal differences in plant N uptake and total N retention. However, knowledge of the effects of N seasonality on the competition between C3 and C4 species is limited. Due to the differences in NUE between C3 and C4 plants (Sage and Pearcy, 1987), the temporal changes in soil N availability could potentially alter the competitive outcomes (Winslow et al., 2003). Interspecific competition may differentially impact plant N-use strategies of C3 and C4 species due to their dramatic differences in response to environmental factors (Halsted and Lynch, 1996; Long, 1999). Better understanding of the plant N responses of C3 and C4 species to N seasonality will help us to understand mechanisms of temporal niche separation between C3 and C4 species and their competition for N resources.

In northern China, C3 and C4 grasses are widely found in the steppe community in Inner Mongolia, which are characterized by seasonal drought and nutrient-poor soils (Yang et al., 2005; Yuan and Zhou, 2005). The productivity of the native steppe is limited by soil N supply (Zhu, 2004). Despite this, little is known about the comparative effects of N seasonality on the N-use strategies of temperate C3 and C4 grasses. In the present study, an experiment altering the temporal distribution of N supply, without changing total N amounts, was designed to investigate the effects of altered N seasonality on plant N-use strategies of C3 and C4 species. Two common grass species (a C3 grass, Leymus chinensis, and a C4 grass, Chloris virgata), representing different photosynthetic pathways in the temperate steppe of northern China, were selected in this study. These two grass species chosen for the study are abundant in this area. Due to the differences in NUE between C3 and C4 species (Sage and Pearcy, 1987), these two species would be expected to differ in response to N seasonality and competition. The specific objectives were to address the following questions. (1) How will the altered N seasonality influence plant N uptake and use? (2) How do the plant N-use traits of C3 and C4 species respond to the interspecific competition? (3) Is the competitive interaction between C3/C4 grass species affected by the altered N seasonality in terms of N-use traits of C3 and C4 species?

**MATERIALS AND METHODS**

**Plant material and growth conditions**

The details of this experiment have been described in Niu et al. (2006). In brief, two species representing different photosynthetic pathways were used. Leymus chinensis (Trin.) Tzvel, a C3 grass, is a widespread and common species in the arid and semi-arid grassland in Inner Mongolia, China. Growing in loose tufts, it is highly tolerant of drought, low fertility and high pH, and is high yielding. Due to extensive clonal growth, its roots, rhizomes and stems form a web in the upper soil, from which branches extend above ground. Chloris virgata Swartz, a C4 grass, is a native species with creeping stems that is common and widely distributed in the Inner Mongolia grassland. The two species often coexist in this area (Sheng and Yang, 2000; Zhao and Sun, 2004). The present study was carried out at the Institute of Botany, the Chinese Academy of Sciences, China (39°54’N, 116°28’E). Precipitation averages 507.7 mm per year, with a high precipitation period in July and August. Mean monthly temperatures range from −3.7 °C in January to 27.3 °C in July, with an annual temperature of 13.0 °C. The relative humidity varied between 52 % and 71 %, with a mean of 61 %.

Seeds of both species were collected from several plants in autumn 2003 in the temperate grasslands in northern China. The smaller and bigger seeds were discarded to homogenize initial seedling weight. Germination tests were conducted to determine the time required for seed germination, in order to be able to transplant both species at the same time. The seeds were germinated in March 2004 in shallow plastic trays covered with a 1–2 mm layer of sand and peat (3 : 1) in a greenhouse at IBCAS, as described previously (Niu et al., 2006). Once the first pair of leaves or photosynthetic cotyledons was fully expanded, seedlings were carefully dug up and then transplanted individually into black polyethylene plastic pots (21 cm diameter, 21 cm height) with a capacity of 4.5 L and filled with 3 kg of sand. The pots were put outside the greenhouse on 21 April 2004 where they received ambient light and precipitation. Eight seedlings were transplanted into each pot and 10 d later six were removed, leaving two strongly growing seedlings.

**Design of nitrogen treatments**

All samples received equal amounts of N. However, three levels of N seasonality were applied: (1) an average model (AM) with N evenly distributed over the growing season; (2) a one-peak model (OM) with more N in summer than in spring and autumn; and (3) a two-peak model (TM) with more N in spring and autumn than in summer (Fig. 1). Nitrogen was supplied at 5-d intervals from 1 May to 28 October. N-mineralization estimates for natural ecosystems in the native habitat of these two grasses range from 0.89 to 1.35 mg N kg⁻¹ month⁻¹ using an incubated-soil-core approach (Wang et al., 2000).

![Fig. 1. Amount of nitrogen applied each time over the growing season.](downloaded from https://academic.oup.com/aob/article-abstract/100/4/821/147745 by guest on 12 March 2019)
The total N amount applied was 100 mg N kg\(^{-1}\) sand for all the three N treatments (see Niu et al., 2006). Nitrogen was supplied in the form of NH\(_4\)NO\(_3\) and P in the form of KH\(_2\)PO\(_3\). Each pot received 1.8 mg N and 0.12 mg P every 5 d. Water was supplied when necessary. Plants were grown during the period 1 May to 30 October 2004. To minimize potential environmental heterogeneity, plants were assigned at random to different N treatments, harvest dates and blocks.

Three competition treatments (a L. chinensis monoculture, a C. virgata monoculture and a mixture of the two species; two individuals per pot) were maintained under the three N seasonality treatments (AM, OM and TM). Between 20 and 30 April 2004, the pots with dead plants (approx. 15 \%) were replaced by spare pots under the same conditions, and the experimental treatments begun. The final experimental design, therefore, was three N seasonality \(\times\) three species combinations \(\times\) six replicates.

**Plant sampling procedure**

Plants were harvested at approx. 15-d intervals, for a period of 6 months. Twelve harvests were performed per species/treatment. Nitrogen concentrations were analysed every month. To analyse the N concentration in dead tissues, the harvest on 30 November was used when the above-ground parts were thoroughly dead. The first harvest (day 0) was carried out 2 weeks after seedlings were transplanted. At each harvest, six replicates per species/treatment were randomly selected. The soil was carefully rinsed off the roots until they were disentangled. Plant material in each pot was divided into above- and below-ground parts. All plant parts were washed, oven-dried at 65°C for 48 h, and weighed to the nearest 0.1 mg. After drying, the tissues were finely ground and total N was analysed by the Kjeldahl acid-digestion method with an Alpkem autoanalyser (Kjektec System 1026 Distilling Unit, Sweden).

**Calculations**

Nitrogen-use efficiency (NUE) was calculated as the product of N productivity (NP) and mean residence time (MRT) of nitrogen according to Berendse and Aerts (1987). There is a presumption that the plant is in a ‘steady state’ with respect to biomass production and nutrient content when calculating NUE components (Berendse and Aerts, 1987; Garnier and Aronson, 1998; Yasumura et al., 2002; Norby and Iversen, 2006; Yuan et al., 2006). Accordingly, NP was calculated for shorter intervals, as proposed by Eckstein and Karlsson (2001) and Yuan et al. (2004), according to the following equation:

\[
NP = \frac{M_2 - M_1}{t_2 - t_1} \cdot \frac{\ln N_2 - \ln N_1}{N_2 - N_1}
\]

where \(M\) and \(N\) are plant biomass and N content size, respectively, in two consecutive harvests conducted at times \(t_1\) and \(t_2\). This equation is based on the assumption that a linear relationship exists between plant biomass and plant N content size, but does not require that NP remains constant between the harvest periods (Evans, 1972). Average NP was calculated as the mean values of the estimated NP(\(t\)) for all harvest periods (Vázquez de Aldana and Berendse, 1997; Eckstein and Karlsson, 2001; Silla and Escudero, 2004). Nitrogen content of living and dead plant parts was calculated using their respective N concentration and the dry mass. Mean annual N content was estimated as a weighed average over the entire year. MRT at the plant level was calculated as the ratio between the average N content and annual N losses (Eckstein et al., 1999; Eckstein and Karlsson, 2001; Norby and Iversen, 2006). Nitrogen losses were calculated based on the N concentration of the above-ground dead material harvested in November plus the difference in above-ground biomass between September and November 2004, since some dead material was lost during the latter harvest due to strong winds. As mentioned by Eckstein et al. (1999), the main flaw in this approach appears to be that the estimate obtained is only representative of the growth period of the study. However, this should not limit the meaningfulness of the MRT for the purposes of comparing plant strategies (Eckstein et al., 1999; Eckstein and Karlsson, 2001; Norby and Iversen, 2006). Additionally, understanding the variations in NUE components is probably more important during the initial stages of growth to explain the dominance or coexistence of species.

Nitrogen concentrations in green and senescing tissues were used to calculate N-resorption efficiency (NRE, \%) = \(\left(\frac{N_{Cg} - N_{Cg}}{N_{Cg}}\right) \times 100\), where \(N_{Cg}\) and \(N_{Cg}\) are the N concentrations in green and senescing tissues, respectively. Nitrogen concentration in senescing tissues was used as a direct indicator of N-resorption proficiency (NRP), i.e. the extent to which N concentrations were reduced in senescent tissues (Killingbeck, 1996; Richardson et al., 2005). The effects of N seasonality and competition (i.e. monocultures and mixtures) and their interactions on individual plant N concentrations, content and NUE were tested for each species by two-way ANOVA, using the General Linear Models (GLM) procedure for analysis of variance with SPSS 11.5 for windows. Data were log\(_{10}\) transformed prior to analysis to meet statistical assumptions.

**RESULTS**

Plant N concentration

Sampling date significantly affected N concentrations in both species \((P < 0.001)\). Under all the three N seasonality treatments, the seasonal patterns of plant N concentrations were similar for both species (Fig. 2), with both above- and below-ground N concentrations decreasing markedly as the growing season went on. There were profound interspecific differences in N concentrations. N concentrations of L. chinensis, on average, were 31 \%, 49 \% and 38 \% higher than those of C. virgata in shoots, roots and total plant, respectively. In addition, shoot N concentrations in both species were higher than those of roots (Table 1).
Significant differences in all shoot, root and total plant N concentrations were observed among the three N treatments in both *L. chinensis* and *C. virgata*, with the highest value of *L. chinensis* under the one-peak model (OM) treatment and *C. virgata* under the two-peak model (TM) treatment, respectively (Tables 1 and 2). Competition impacted N concentration in *L. chinensis* ($P < 0.001$). When compared with being in a monoculture, *L. chinensis* in a mixture tended to have lower N concentrations in shoots (14 %) and whole plants (12 %), but similar root N concentrations. By contrast, no differences in either shoot or root N concentrations in *C. virgata* were observed between monoculture and mixture ($P > 0.05$; Tables 1 and 2). There were also significant effects of interactions between N seasonality and competition on N concentrations in shoots, roots and whole plants in *L. chinensis* as well as on shoot N concentrations in *C. virgata* (Table 2).

### Plant N content

There was strong temporal variability in N contents in both species over the growing season ($P < 0.001$). Nitrogen contents in both shoots and roots increased rapidly at the start of the growing season, with maximum

### Table 1. Mean N concentrations and contents of *L. chinensis* and *C. virgata* over the growing season under the three N treatments

<table>
<thead>
<tr>
<th>Species</th>
<th>N treatment</th>
<th>N concentration (mg g$^{-1}$)</th>
<th>N content (mg plant$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td><em>L. chinensis</em></td>
<td>Monoculture</td>
<td>AM</td>
<td>14.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OM</td>
<td>15.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TM</td>
<td>14.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Mixture</td>
<td>AM</td>
<td>11.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OM</td>
<td>12.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TM</td>
<td>13.2 ± 0.2</td>
</tr>
<tr>
<td><em>C. virgata</em></td>
<td>Monoculture</td>
<td>AM</td>
<td>10.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OM</td>
<td>9.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TM</td>
<td>11.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Mixture</td>
<td>AM</td>
<td>10.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OM</td>
<td>10.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TM</td>
<td>10.3 ± 0.3</td>
</tr>
</tbody>
</table>

Data are presented as means ± s.e. ($n = 6$).

AM, Average model; OM, one-peak model; TM, two-peak model.
Nitrogen productivity of NP and NUE, but lower MRT than species had the lowest NP, MRT and NUE under the OM significantly affected NP, MRT and NUE (Table 3). This C. virgata mainly due to a higher dry matter production in higher than that of L. chinensis (Fig. 4). The difference in NP was as well as \textit{C. virgata} had higher N contents under the AM treatment (Tables 1 and 2). Significant interactions of N seasonality and competition were observed on any of the three variables were detected in either species.

NP, MRT and NUE

There were significant differences in N-use strategies between the two grass species. \textit{Chloris virgata} had higher NP and NUE, but lower MRT than \textit{L. chinensis} (Fig. 4). Nitrogen productivity of \textit{C. virgata} was, on average, 30 % higher than that of \textit{L. chinensis}. The difference in NP was mainly due to a higher dry matter production in \textit{C. virgata}. For \textit{L. chinensis}, the altered N seasonality significantly affected NP, MRT and NUE (Table 3). This species had the lowest NP, MRT and NUE under the OM treatment. Competition also impacted NP, MRT and NUE in \textit{L. chinensis} (\(P < 0.001\)). \textit{Leymus chinensis} had higher MRT, by an average of 17 %, in the mixture than in the monoculture. Nitrogen productivity and thus NUE in \textit{L. chinensis} were also higher in the mixture than in the monoculture (Fig. 4).

For \textit{C. virgata}, N seasonality treatments influenced NP only (\(P < 0.01\); Table 3). There were no differences in MRT or NUE among the three N seasonality treatments. Nevertheless, both NP and use efficiency showed the greatest values under the OM treatment (Fig. 4). Competition had significant effects on NP, MRT and NUE in \textit{C. virgata} (\(P < 0.05\)). All the three parameters in \textit{C. virgata} were higher in the mixture than in the monoculture. No interactive effects of N seasonality and competition on any of the three variables were detected in either species.

NRE and NRP

There were interspecific variations in these two indices (Fig. 5). \textit{Chloris virgata} had higher NRE and NRP than \textit{L. chinensis} in both the mixture and the monoculture (\(P < 0.01\)). Both NRE and NRP differed significantly among the three N seasonality treatments for the two grass species (Table 3). \textit{Leymus chinensis} had the lowest NRE, but the highest NRP under the OM treatment. By contrast, \textit{C. virgata} had the highest NRE, but the lowest NRP under the OM treatment. Competition impacted these two resorption indices, with higher NRE and lower NRP in the mixture than in the monoculture (Table 3).

DISCUSSION

Temporal variability and interspecific differences

A declining trend in plant N concentration and an increasing trend in plant N content were observed in both the C₃ (\textit{L. chinensis}) and C₄ (\textit{C. virgata}) species during the growing season (Figs 2 and 3), which could be attributable to the dilution effect and the accumulative effects of plant N uptake, respectively. The tendency of N concentration to decrease with time is primarily the result of an increase in plant biomass, reflecting the increasing proportions of structural and storage materials containing little N (Greenwood et al., 1990). Therefore, seasonal changes in N concentration in plants result directly from growth-dilution effects. Seasonal trends in biomass and N concentrations of shoots and roots (Fig. 2) showed that, in general, from late-summer onwards the proportion of total N in the shoot declined while the proportion in the rhizome increased, suggesting that efficient internal recycling of N occurred in both species. An alternative explanation for the decline in N concentration in the above-ground components could be leaching.

Consistent with previous studies, profound differences in N concentration, productivity and N resorption indices were found between the C₃ \textit{L. chinensis} and the C₄ \textit{C. virgata} grass species. In comparison with \textit{L. chinensis}, higher NUE in \textit{C. virgata} allowed this species to allocate more

<table>
<thead>
<tr>
<th>Species</th>
<th>N (2 d.f.)</th>
<th>C (1 d.f.)</th>
<th>N × C (2 d.f.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{L. chinensis}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot N concentration</td>
<td>6.849**</td>
<td>134.407***</td>
<td>13.216***</td>
</tr>
<tr>
<td>Root N concentration</td>
<td>5.454*</td>
<td>8.388 NS</td>
<td>7.000***</td>
</tr>
<tr>
<td>Total N concentration</td>
<td>7.187**</td>
<td>122.477***</td>
<td>6.567**</td>
</tr>
<tr>
<td>Shoot N content</td>
<td>11.876***</td>
<td>1472.515***</td>
<td>3.682**</td>
</tr>
<tr>
<td>Root N content</td>
<td>36.872***</td>
<td>1251.521***</td>
<td>1.294 NS</td>
</tr>
<tr>
<td>Total N content</td>
<td>26.512***</td>
<td>2377.761***</td>
<td>4.176*</td>
</tr>
<tr>
<td>\textit{C. virgata}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot N concentration</td>
<td>8.118**</td>
<td>2.129 NS</td>
<td>4.164*</td>
</tr>
<tr>
<td>Root N concentration</td>
<td>6.503**</td>
<td>1.198 NS</td>
<td>0.765 NS</td>
</tr>
<tr>
<td>Total N concentration</td>
<td>8.067**</td>
<td>8.020**</td>
<td>0.996 NS</td>
</tr>
<tr>
<td>Shoot N content</td>
<td>61.366***</td>
<td>4.699*</td>
<td>16.833***</td>
</tr>
<tr>
<td>Root N content</td>
<td>33.807***</td>
<td>10.241***</td>
<td>6.584*</td>
</tr>
<tr>
<td>Total N content</td>
<td>74.509***</td>
<td>1.733 NS</td>
<td>18.492***</td>
</tr>
</tbody>
</table>

Significance levels are indicated by *** \(P < 0.001\); ** \(P < 0.01\); * \(P < 0.05\); NS, not significant.

d.f., Degrees of freedom.
resources (including N) to the above-ground part in acquiring more light resources. Differences in N dynamics and N-use strategies could have significant influences on photosynthesis, growth and competition of these two plant functional types, with consequent impact on their distribution and dominance in natural conditions.

Responses to changed nitrogen seasonality

It is widely accepted that greater soil N availability leads to higher plant N concentrations (Aerts and Chapin, 2000). The present observations showed that temporal fluctuations in soil N availability could also affect plant N concentrations even with equivalent amounts of N supply (Fig. 2 and Table 1). Under the OM treatment, the greater N supply in summer combined with a warmer environment might have enhanced N uptake in L. chinensis, and thus plant N concentration. By contrast, higher N supply in the early and later growing season may be of benefit to N uptake and concentration of C. virgata. Plant N content was also affected by the altered N seasonality. However, due to different responses in plant biomass to treatments, higher N concentration does not necessarily lead to greater net N accumulation at the shoot, root and whole plant levels, especially for the C3 species, L. chinensis, which had the highest N contents under the AM treatment in both monoculture and mixture. The above results suggest differential responses in plant N concentration and biomass growth to N seasonality treatments in L. chinensis.

NUE, the ratio between biomass production and N loss in senesced tissues (Vitousek, 1982), represents the effectiveness of plants in producing biomass per unit of N uptake. Thus, NUE is associated with tissue chemistry and N resorption from senescing tissues (Eckstein et al., 1999;
In this study, average resorption efficiency of the study species between 30% and 61% (Fig. 5) matched values found for a large number of different plant species (Killingbeck, 1996; Aerts and Chapin, 2000; Richardson et al., 2005; Yuan et al., 2005). Similar to N concentration and content, the altered N seasonality significantly impacted all the N-use and resorption variables in both species except for MRT and NUE in C. virgata (Table 3).

It has been well documented that, compared with C₄ species, photosynthesis of C₃ species is greater under low temperatures in spring and autumn and lower under high temperatures in summer (Kemp and Williams, 1980; Niu et al., 2005, 2006). The different seasonal patterns between C₃ and C₄ species in response to temperature variability have been the fundamental presumption for niche separation between the two functional types (Kemp and Williams, 1980; Ode et al., 1980). In the present study, the seasonal pattern of N supply under the one-peak model treatment (OM, with more N in summer and less in spring and autumn) coincided with the seasonal patterns of temperature, but opposite to the seasonal dynamics of photosynthesis of C₃ species. Interestingly, all NP, MRT, NUE and NRE values of the C₃ grass species (L. chinensis) under the one-peak model treatment were lowest among the three N treatments (Figs 4 and 5). By contrast, the seasonal pattern of N supply under the one-peak model followed the seasonal dynamics of photosynthesis of C₄ species (i.e. higher in summer and lower in spring and winter). Consequently the greatest N-use and resorption variables (except for NRP) of the C₄ grass species (C. virgata; Figs 4 and 5) were observed. Given the well-documented positive relationships between plant photosynthesis with leaf N concentration, the greater N supply under the one-peak model has, therefore, met the greater requirement of N uptake for the higher photosynthetic capacity of C₄ species in summer, consequently leading to improved NUE under this treatment. The observations suggest that N seasonality plays an important role in regulating plant growth and NUE.

**Plant responses to competition**

The C₃ species (L. chinensis) and the C₄ species (C. virgata) grow together in the natural grassland ecosystems in northern China. In this experiment, L. chinensis growing in a mixture accumulated less biomass than plants growing in a monoculture whereas no competitive

**Table 3. The effects of nitrogen seasonality (N) and competition (C) on variables related to plant productivity and N turnover in the C₃ grass L. chinensis and the C₄ grass C. virgata**

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>L. chinensis</th>
<th>C. virgata</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP</td>
<td>0.0007</td>
<td>0.0011</td>
</tr>
<tr>
<td>MRT</td>
<td>0.0382</td>
<td>0.5134</td>
</tr>
<tr>
<td>NUE</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NRE</td>
<td>&lt;0.0001</td>
<td>0.0095</td>
</tr>
<tr>
<td>NRP</td>
<td>0.0095</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

Numbers are P-values obtained from analysis of variance. Significant values (P < 0.05) are in bold.

NP, N productivity; MRT, mean residence time of N; NUE: N-use efficiency; NRE, N-resorption efficiency; NRP, N-resorption proficiency.
could partly explain the competitive results between the two plant species, the competitive responses of NUE and its components (NP and MRT) were not related to the changes in biomass of the two species under competition. The results suggest that in most natural ecosystems, competition between the two species is not only for nutrients, but also for other resources, e.g. light resources in this study.

Although the present study examined only one species from each functional group, the results are consistent with the hypothesis that both N seasonality and competition may impact N-use strategies of \( C_4 \) and \( C_3 \) grasses. \textit{Leymus chinensis} and \textit{Chloris virgata} use the \( C_3 \) and \( C_4 \) photosynthetic pathways, respectively. The contrasting effects of N treatments on these species’ N economy may be due to differences in the performance of the two functional groups. \textit{Chloris virgata} used N more efficiently than \textit{L. chinensis} for biomass production. \textit{Leymus chinensis} initially grows slowly because it is unable to produce a large photosynthetic surface quickly as the result of its small, thick leaves containing a relatively large dry mass per unit area (Zhu, 2004). \textit{Leymus chinensis} leaves had smaller area per unit mass, suggesting that they require more resources for construction than \textit{C. virgata} leaves. \textit{Chloris virgata} has a much larger foliar biomass compared with \textit{L. chinensis} (S. Wan et al., unpubl. res.). Because foliage is rich in N compared with other tissues, the larger foliar biomass of \textit{C. virgata} would create a higher N demand. The \( C_4 \) species \textit{C. virgata} in both monoculture and mixture had higher NUE values than the \( C_3 \) species \textit{L. chinensis} (Fig. 4) due to higher photosynthetic rates for \textit{C. virgata} (Niu et al., 2005, 2006). Because NUE increases with soil N deficiency (Knops et al., 1997; Pastor and Bridgham, 1999), the higher NUE of the \( C_4 \) species in both a monoculture and a mixture under altered water and N seasonality suggested that \textit{C. virgata} may be less limited by soil N than \textit{L. chinensis}, although both species grew on soils with the same N availability. Differentiation in resource use may help to maintain species diversity and minimize competition for resources in some plant communities (Tilman, 1982).

Because photosynthetic NUE is a major determinant of NP (Garnier et al., 1995), the dominant species \textit{C. virgata} in mixture had higher NP than the subordinate species \textit{L. chinensis} (Fig. 4), in part because of its higher photosynthetic NUE (Niu et al., 2005). Both plant lifespan and N resorption efficiency can impact on MRT (Eckstein et al., 1999; Aerts and Chapin, 2000). The subordinate species had lower NRE than the dominant species (Fig. 5) and thus the significantly longer MRT in the subordinate species should not be ascribed to resorption efficiency. In the present study, however, \textit{L. chinensis} had a longer lifespan (6 months) than \textit{C. virgata} (5-6 months) in the field. Thus the longer MRT in \textit{L. chinensis} can be explained by its longer lifespan.

Greenhouse experiments may not always be directly applicable to a natural community because of differences in temperature and moisture conditions, duration of the growth period, reproduction of grasses from seed rather than vegetative shoots, and reductions in growth caused by small soil volumes. The results may be species specific.
Under drought conditions the biomass of both the C3 species *L. chinensis* and the C4 species *C. virgata* was less than in field conditions (data not shown). C4 species have higher water-use efficiency as well as higher temperature optima for photosynthesis, and are more abundant in a warm, dry climate (Teeri and Stowe, 1976). Further, the present experiment was conducted in Beijing, where the meteorological conditions, including precipitation, temperature, light and wind, differ to a certain extent from Inner Mongolia. In addition, the amount of N added was higher than the net N mineralization observed in the field (see the Materials and methods). Therefore the results from the present study may not be valid for other ecosystems.

**CONCLUSIONS**

In this paper, the N uptake and use of one C3 and one C4 grass species under altered N seasonality were investigated. The results of this study support the hypothesis that C3 grasses are nutritionally superior to C4 grasses; N concentration is, on average, higher in a C3 grass than in a C4 grass. The altered N seasonality also changed plant N-uptake and use-strategies. Therefore, the intra-annual variability in soil N supply may be an important regulator of plant N-use strategies and the possible niche separation between C3 and C4 species. Furthermore, competition affected plant NUE, which may change the interactions between C3 and C4 species.

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**LITERATURE CITED**


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