



## Leaf nitrogen is closely coupled to phenophases in a desert shrub ecosystem in China



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### ABSTRACT

The understanding of the seasonal dynamics of leaf nitrogen (N) relative to leaf traits for desert shrub species is limited. We investigated the seasonal changes in leaf N, soil N, specific leaf area (SLA) and photosynthetic capacity ( $P_{max}$ ) in relation to the phenology of *Artemisia ordosica* in a desert shrubland area in northern China during the growing season of year 2012. We found that the amount of leaf N per unit dry mass varied in different phenological phases, having higher values during the early leaf expansion and leaf coloration and early defoliation periods and lower values during the expanded leaf and flowering periods.  $P_{max}$  was positively related to leaf N from the late leaf expansion period to the flowering and bearing period. Photosynthetic nitrogen use efficiency (NUE) correlated with SLA during the growing period. However, similar relationships were not observed for the leaf expansion and defoliation periods. During the flowering and bearing period, leaf N content per unit dry mass remained relatively stable, although fluctuations occurred due to precipitation. Our findings could help with modelling the dynamics of plant-soil N coupling of desert shrub-land ecosystems under changing environment.

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### 1. Introduction

Plant traits such as specific leaf area (SLA) and leaf nitrogen concentration (N) play a pivotal role in the light capture, CO<sub>2</sub> assimilation and carbon sequestration of plants (Rozendaal et al., 2006; Gelfand et al., 2012). Leaf N is strongly correlated with the rate of photosynthesis in the leaf. A shortage of leaf N may limit plant growth, as most of leaf N is invested in photosynthetic enzymes (Reich et al., 1997; Goedhart et al., 2010). SLA is also closely related to photosynthetic nitrogen use efficiency (NUE) (Schieving and Poorter, 1999). Thus, SLA and NUE can be used together to estimate the seasonal and annual photosynthetic capacity and carbon sequestration potential of plants (Reich et al., 1991, 1992). However, the possible acclimation of photosynthesis to a changing environment may also depend on the responses of SLA and NUE to environmental changes (Rosati et al., 1999; Benomar et al., 2011).

There may also be differences in species-specific responses to changes in nitrogen availability. An understanding of these differences is crucial in predicting ecosystem feedback in relationship to climate change (Araya et al., 2010; Benomar et al., 2011; Butler et al., 2012).

Leaf N concentration is also affected by availability of soil N, capacity of the plant for N absorption, and partitioning of N among plant tissues (Wright et al., 2005; Taylaran et al., 2011). The availability of soil N directly influences a wide range of above- and below-ground ecological processes at the individual, population, community, and ecosystem level (Frank and Groffman, 2009). However, plants may also have species-specific impacts on soil nutrient availability through feedback mechanisms involving the nutrient cycle (Ordoñez et al., 2009).

The understanding of effects of a changing environment, e.g., changes in soil and plant processes in desert ecosystem is still limited (Orwin et al., 2010). According to Butler et al. (2012), soil warming may induce an increase in available soil N, which may consequently increase leaf N and the relative growth rate. NUE may also be positively related to soil N (Osada et al., 2010). However,

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increasing atmospheric CO<sub>2</sub> concentration may decrease availability of N in the soil and decrease leaf N (Kahmen et al., 2008; Garten et al., 2011). Such environmental changes and variations in soil N may also cause plants to adapt by changing their traits (Crous et al., 2010).

N deposition through precipitation may contribute significantly to soil N in desert ecosystems, but their relationship is still unclear for the following reasons: soil N levels are low and heterogeneous in space and time, soil N levels are frequently masked by soil water deficiencies (Austin and Vitousek, 2000; Aranibar et al., 2004), and the N stores are concentrated at the soil surface and underneath shrub canopies (Schlesinger and Pilmanis, 1998; Aranibar et al., 2004).

In previous studies, the relationships between soil N, leaf N, SLA, photosynthetic capacity and ecosystem NUE during various phenological phases have primarily been examined in forest (e.g., Garten et al., 2011) and grassland ecosystems (Kahmen et al., 2008; Lu et al., 2012). However, an understanding of these relationships is also needed for desert shrublands (McDonald et al., 2003; Lü et al., 2012).

Overall, seasonal changes in leaf N are a common consequence of plant genetic characteristics and environmental responses (Bresinsky et al., 2013). In this study, we hypothesized that the seasonal dynamics of leaf N would be dependent on phenology and availability of soil N which is associated with precipitation, thus having an influence on ecosystem photosynthetic capacity. To verify our hypotheses, we investigated the seasonal changes in leaf N of *Artemisia ordosica*, one of the principal shrub species of desert shrubland in northern China. The major aims of the study were: (1) to examine changes in leaf and soil N in response to phenology and precipitation and (2) to determine the temporal responses of photosynthetic capacity to N dynamics both in leaf and soil.

## 2. Materials and methods

### 2.1. Site description

The measurements for this study were performed at Yanchi Research Station of Beijing Forestry University, Ningxia, China (37.68°–37.73°N, 107.20°–107.26°E). The site is located between the arid and semi-arid climatic zones at the edge of the Mu Us desert at an elevation of ~1550 m a.s.l. The soil at the site is sierozem with >70% fine sand (0.02–0.2 mm). The principal habitats in this area are shifting sand dune, semi-fixed dune and fixed dune. The prevailing climate is of the temperate arid and semi-arid type. The mean annual precipitation was 292 mm, of which 62% fell between July and September. The mean annual total potential evapotranspiration was 2024 mm. The mean annual temperature was 8.1 °C. All meteorological data for the study area were provided by the meteorological station of Yanchi County and represented 51-year averages (1954–2004). The vegetation is naturally developed shrubland. The principal shrub species is *Artemisia ordosica*.

### 2.2. Phenological and plant observations

A 100 m × 100 m plot was established around an eddy-covariance flux tower at the site. The plot is flat and the dominant vegetation was *Artemisia ordosica*, with 13.3% coverage which accounted for 90% of its total. Accordingly, the site is considered a monospecies site. The plot was divided into 20 m × 20 m grids. Twelve 10 m × 10 m subplots were established in every other grid in the plot. Each subplot was located in southeast corner of a grid. Three plants with plot-averaged height were randomly selected in each subplot for the observation of phenophases over the growing season. The definition of phenophases were described in Table 1.

For leaf area measurement, ten fascicles of outer canopy leaves were collected on three selected plants from April to October 2012 in each of 12 subplots every seven days except during June, July, and August, when the sampling interval was ten days. For all leaf samples, the leaf area of fresh leaves was measured with an LI-3100C leaf area meter (LI-COR Environmental, Lincoln, Nebraska, USA). The SLA was calculated as the ratio of fresh leaf area to dry mass (oven-dried at 70 °C for 48 h). The leaf samples of three plants from each subplot were then combined for N concentration analysis using the Kjeldahl method. Altogether, therefore, 12 pooled leaf samples from each sampling time were used for N analysis in the laboratory. The leaf N concentration was determined both on a dry weight basis ( $N_{\text{mass}}$ , g N kg<sup>-1</sup> dry weight) and on a leaf area basis ( $N_{\text{area}}$ , g N m<sup>-2</sup> leaf area).

The leaf area index (LAI) of the study area was measured once every week using a plant canopy analyzer (LI-2000; LI-COR, Lincoln, NE, USA) at 36 fixed points around the eddy flux tower. The 36 points for periodic LAI measurement were positioned at 36 evenly distributed grid (20 m × 20 m) intersections in a 100 m × 100 m plot. LAI was usually measured once every week. The LAI used for further analysis was the average of the 36 measurements.

Soil samples were collected using a metallic corer at regular intervals of 15 days. One sample being a mixture of two subsamples with one on each diagonal of a subplot from the upper 30 cm of the soil profile, was taken after removing the leaf litter layer on each sampling date. Samples for each subplot over the season were taken with 1-m interval along diagonal of the subplot. There were totally 12 soil samples on each sampling date. The soil samples were air-dried for two weeks, sieved (<2 mm mesh size) to remove stones, large roots and non-decomposed leaf litter, and then stored in ziplock bags in a cool dry place for total N concentration ( $N_{\text{mass}}$ ) analysis using the Kjeldahl method.

### 2.3. Eddy covariance measurements

Net ecosystem CO<sub>2</sub> exchange (NEE) was measured continuously at 4.5 m above ground level using the eddy covariance (EC) technique. The EC system consisted of a 3D sonic anemometer (WindMaster™ Pro, Gill Instruments Ltd, Lymington, England) and closed-path infrared CO<sub>2</sub>/H<sub>2</sub>O gas analyzer (model LI-7200, LI-COR, Lincoln, NE, USA). EC measurements were taken at a frequency of 10 Hz, and turbulent fluxes were recorded on a datalogger (CR3000, Campbell Scientific) each half hour.

Meteorological variables were measured next to the EC system. Photosynthetically active radiation (PAR) was measured with a radiation sensor (LI-190SA, LI-COR, Lincoln, NE, USA) at 4 m above the ground. A tipping bucket rain gauge (model TE525MM, Campbell Scientific Inc., Logan, UT, USA) 1 m above the ground was used to measure precipitation. Soil volumetric water content (VWC) was measured with soil moisture sensors (5 TE, Decagon Devices, USA) at depths of 0.1, 0.3 and 0.7 m in three pits. Data from these sensors were recorded using a digital datalogger (EM50, Decagon Devices, USA).

### 2.4. Statistical analysis

The light-saturated rate of net CO<sub>2</sub> assimilation ( $P_{\text{max}}$ ) was estimated twice per month from the light response curve of net ecosystem production (NEP) based on Equation (1) (Lambers and Chapin III, 2008). Half-hourly NEP and PAR values from daytime periods were fit to Equation (1) (Michaelis-Menten, 1913; Baly, 1935).

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