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Grass–legume ratio can change soil carbon and nitrogen storage in a temperate steppe grassland

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ABSTRACT

In grassland, introduction of legume potentially can play a significant role in increasing soil carbon (C) and nitrogen (N) storage through increasing biomass C input. It has been reported that grass–legume ratio (GLR) is important in assessing benefits of grass–legume mixtures, however, little is known about how GLR affects soil C and N storage. As an effort to understand the effects and mechanisms of GLR on soil carbon and nitrogen storage, biological nitrogen fixation (BNF) by legumes, and changes in C and N storage in plants and soil were examined under swards with different densities of grasses and legumes over 4 years in a temperate steppe grassland. Results showed that total N storage in 0–40 and overall 0–150 cm soil depths varied as GLR changed, reaching peak values when the GLR was equivalent to 1:1. Increased relative legume abundance significantly enhanced soil C storage in both 0–40 and 0–150 cm soil depths, which was primarily attributed to an increase of aboveground biomass C. Soil organic C and total N varied with GLR changes, leading to stoichiometric shifts in soil C:N ratios, possibly influencing soil C storage through changing plant community structure and soil biochemistry. It is suggested that introducing legumes in steppe grasslands with a GLR of 1:1 could increase resources use efficiency and improve the sustainability of the environment.

1. Introduction

About half of the world's land area is occupied by grassland soils which store more than 10% of terrestrial biomass carbon (C) (Scurlock and Hall, 1998). Enhanced grassland soil C storage is linked to increase in biomass C input, thus is limited by soil fertility (Gill et al., 2006). The addition of nitrogen (N) fertilizers has been shown to directly increase soil fertility. However, caution is needed when using mineral fertilizers to increase productivity for a number of reasons. First, N fertilization can cause an increase of additional greenhouse gas (GHG) emissions including nitrous oxide (Mortenson et al., 2004). Second, mineral N fertilizers do not benefit all grassland species equally due to specific differences in aquisition and utilization of nutrients (Suding et al., 2005). Thus, historical and experimental evidence has indicated that chronic or intensive N fertilization can reduce plant species diversity (Bai et al., 2010; Clark and Tilman, 2008; Suding et al., 2005).

As an essential component of grassland, legumes are playing an important role in producing high quality forage due to their high N and protein content (Mortenson et al., 2004). More importantly, legumes can improve soil fertility through biological N fixation (BNF) (Herridge et al., 2008; Paynel et al., 2008; Spehn et al., 2002), thus helping in maintaining high dry matter and N yield in grasslands (Frankow-Lindberg and Dahlin, 2013; Nyfeler et al., 2011). Legumes also have considerable additional benefits beyond their importance regarding N fixation and high protein level. These include positive effects on reducing GHG emissions and increasing biodiversity (Abberton, 2010). Some forage legumes possess secondary metabolites known as condensed tannins (CTs) in plant tissues, which can help reduce methane production in ruminants (Ramirez-Restrepo and Barry, 2005). Additionally, introducing legumes as N donators to replace mineral fertilizers can reduce the GHG emissions from soil and fertilizer production systems (Gregorich et al., 2005). Compared with N fertilization, N fixed by legumes can provide a more slow-released and persistent N source. This may reduce the competitive advantage of more efficient N consuming species which can be advantageous for maintaining higher species diversity and increasing resource complementarity (van Ruijven and Berendse, 2005). Therefore,

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legumes can play a potentially significant role in reducing N fertilizers to increase biomass production, consequently enhancing carbon storage of soil in grasslands.

Research indicates that legume introduction significantly increases soil C and N accumulation in grasslands (Fornara and Tilman, 2008; Mortenson et al., 2004). Relevant mechanisms were also proposed to explain the change of soil C and N with presence of legumes (Fargione et al., 2007; Fornara and Tilman, 2008). However, the benefits from grass–legume mixtures depend on the trade-off with intra- and inter-specific competition and facilitation effects (Lithourgidis et al., 2006). The grass–legume ratio can impact community structure and function through altering intra- and inter-specific interaction in mixture grasslands (Kirwan et al., 2007; Nyfeler et al., 2011). It has been reported that the relative density of legumes in mixed grasslands influences BNF and community productivity (Kirwan et al., 2007; Ledgard and Steele, 1992; Nyfeler et al., 2011). However, little is known on how grass–legume ratios influence carbon and nitrogen storage in soils. In this study, we explored effects and mechanisms of grass–legume ratios on carbon and nitrogen storage in soils by manipulating the initial density of grasses and legumes in a temperate steppe grassland. We hypothesized that (a) decreasing GLR increased soil N storage because of increased legume abundance and BNF, (b) decreasing GLR enhanced soil C storage as increased BNF and soil N level increased productivity and biomass C input.

2. Materials and methods

2.1. Study site

A field experiment was established at the Grassland Farming Research Station (E123°31′, N44°33′, Elevation 145 m) of the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, which is located at Jilin Province, northeast China. The study site has a semi-arid, continental climate. Mean annual temperature is 4.9 °C, and annual precipitation is approximately 410 mm, with 70% falling from June to September. The soil type is meadow chernozem soil with a pH 8.1, bulk density 1.48 g cm^{-3},organic matter 16 g $kg^{-1}$and total N 1.1 g kg^{-1}at the depth of 0–30 cm. The mature vegetation type in this study site is meadow steppe dominated by *Leymus chinensis*, a perennial C_3 rhizome grass, which accounts for 80% of the above-ground biomass. However, from the 1980s, part of the *L. chinensis* meadow had been reclaimed as cropland due to the increasing population pressure. The current experiment was conducted in a section of the meadow that was converted to cropland in 1995, but subsequently abandoned in 2002.

2.2. Field experiment design

Leymus chinensis and *Medicago sativa* were sown into 5 mixes with grass–legume ratios (GLRs) to 1:0 (grass monoculture, 1G0L), 3:1 (3 grass:1 legume, 3G1L), 1:1 (1 grass:1 legume, 1G1L), 1:3 (1 grass:3 legume, 1G3L) and 0:1 (legume monoculture, 0G1L) with corresponding legume contents as 0, 25%, 50%, 75% and 100%, respectively. The target total plant density (combination of grass and legume) was 600 plant individuals m^{-2} which reflected the mean plant density in this *L. chinensis* steppe meadow. A completely randomized block design was employed with 4 replicates. The plot size was 3 m × 3 m.

On 5 July 2006, seeds were mixed as per treatments and uniformly sown into plots with row spacing of 15 cm. All legume seeds were soaked in an acid solution(98% H_2SO_4) for half an hour to soften the seed coat prior to sowing. Plots were irrigated when necessary to ensure emergence and survival of the sown species. On 5 August 2006, plots were thinned to designated target plant densities. No other management was conducted in all plots during the experiment.

2.3. Plant sampling and analysis

In early September 2010, aboveground plant materials were harvested using a 1 m × 1 m quadrat. Plant numbers were counted and identified to species in each quadrat. The aboveground biomass of each species was clipped at the soil surface and taken back to the laboratory using paper bags. All standing and falling litter also was collected in each quadrat. The belowground biomass was collected using a soil auger with 10 cm diameter. Three soil-root samples were taken from each quadrat and then bulked into a composite sample at depths of 0–10, 10–20, 20–40, 40–60, 60–100 and 100–150 cm. Roots were washed free from the soil. All plant materials were oven-dried at 65 °C for 48 h and weighed to determine the dry weight. Both shoot and root materials were finely ground, and analyzed for organic C and total N content using the $K_2Cr_2O_7$ method (Page, 1982) and the Kjeldahl method (Sparks et al., 1996), respectively. Subsamples of legume shoot were finely ground for analyzing ^{15}N natural abundance using a continuous flow Isotope Ratio Mass Spectrometer (Stable Isotope Facility, UC Davis). *Leymus chinensis* in grass monocultures was used as the reference plant (Frankow-Lindberg and Dahlin, 2013; Nyfeler et al., 2011).

2.4. Soil sampling and analysis

Three additional soil samples were taken using an auger with 5 cm in diameter and then bulked into a composite sample at depths of 0–10, 10–20, 20–40, 40–60, 60–100 and 100–150 cm in each quadrat. With visible plant materials and other debris removed, the soil samples were air-dried in the dark, then ground to pass through a 2 mm sieve for chemical analysis. Soil bulk density (BD) for each depth in each quadrat was determined using the core method (Klute, 1986). Soil organic carbon (SOC) was measured using the $K_2Cr_2O_7$ method (Page, 1982). Soil total nitrogen was measured by the Kjeldahl method (Sparks et al., 1996). All soil nutrient traits were calculated based on units of dry soil mass.

2.5. Calculations

2.5.1. Biological nitrogen fixation and nitrogen transfer

Biological nitrogen fixation (BNF) of *M. sativa* was estimated using the ^{15}N natural abundance method. The percent of N derived from the atmosphere ($\%N_{dfa}$) in biomass of *M. sativa* was estimated using the following formula (Unkovich et al., 2008):

$$\%N_{dfa} = 100 \times \left(\frac{\delta^{15}N_{\text{reference plant}} - \delta^{15}N_{\text{legume}}}{\delta^{15}N_{\text{reference plant}} - B} \right) \quad (1)$$

The *B*-value for *M. sativa* was determined by growing them under N-free medium (sand + perlite + nutrient solution) inoculated with a soil suspension from the experimental site in temperature-controlled glasshouse, and harvesting shoot material for ^{15}N analysis at a stage of growth that is the same as our field analyses(Unkovich et al., 2008). Total BNF was calculated based on $\%N_{dfa}$, legume shoot biomass and shoot N concentration.

The contribution of *M. sativa*-derived N ($\%N_{trans}$) to *L. chinensis* in mixtures was estimated according to the following formula (Snoeck et al., 2000):

$$\%N_{trans} = 100 \times \left(\frac{\delta^{15}N_{L.chinensis\text{in monoculture}} - \delta^{15}N_{L.chinensis\text{in mixture}}}{\delta^{15}N_{L.chinensis\text{in monoculture}} - B} \right) \quad (2)$$