Strong stoichiometric resilience after litter manipulation experiments; a case study in a Chinese grassland

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Abstract

Global climate change has generally modified net primary production (NPP) which leads to increasing litter inputs in some ecosystems. Therefore, assessing the impacts of increasing litter inputs on soil nutrients, plant growth and ecological carbon (C):nitrogen (N):phosphorus (P) stoichiometry is critical for an understanding of C, N and P cycling and their feedback processes to climate change. In this study, we added plant above ground litter, harvested near the experimental plots, to the 10-20 cm subsoil layer of a steppe community at rates equivalent to annual litter input of 0, 15, 30, 60 and 120%, respectively, covering the entire range of the expected NPP increases in this region due to climate change (10-60%). We measured the resulting C, N and P content of different pools (above and below ground plant biomass, litter, microbial biomass). Small litter additions, which are more plausible compared to the expected increase predicted by Earth system models, had no effect on the variables examined. Nevertheless, high litter addition (120% of the annual litter inputs) significantly increased soil inorganic N and available P, aboveground biomass, belowground biomass and litter. Our results suggest that while very high litter addition can strongly affect C:N:P stoichiometry, the grassland studied here is resilient to more plausible inputs in terms of stoichiometric functioning.

Keywords: litter addition; C, N and P pools; C:N:P stoichiometry; soil microorganisms; temperate grassland

1. Introduction

Ecological stoichiometry is the study of the balance of multiple chemical elements in ecological interactions (Elser et al., 2000, 2010; Elser and Hamilton, 2007). Carbon (C), nitrogen (N) and phosphorus (P) are key elements in terrestrial ecosystems (Daufresne and Loreau, 2001; Elser et al., 2010; Hessen et al., 2013), and the C:N:P stoichiometry reflects complex interactions between evolutionary processes coupled to phenotypic plasticity (Sardans et al., 2012). These complex interactions are, at least partially, controlled by patterns of element supply from the environment (Hessen et al., 2004). Ecological stoichiometry provides a valuable approach in assessing possible changes in C, N and P cycling (Hessen et al., 2013). Over the past three decades ecological stoichiometry has expanded greatly (Hessen et al., 2013), and many studies have concentrated on understanding the variation in plant C, N and P concentrations among and within species (Schmidt et al., 1997; Aerts and Chapin, 2000; Güsewell and Koerselman, 2002; Augustine et al., 2003; Güsewell 2004; Frank, 2008). However, relationships among soil nutrient availability, plant growth and ecological C:N:P stoichiometry of plants, litter, soil and soil microbes under global climate change in terrestrial ecosystems remain poorly understood.

Litter is an important nutrient pool and litter decomposition rates strongly affect nutrient availability in terrestrial ecosystems with significant feedback on plant growth and on climate (Subke et al., 2004; Ryan and Law, 2005; Sayer, 2006; Cornelissen et al., 2007; Villalobos-Vega et al., 2011). The increase of atmospheric carbon dioxide (CO_2) concentration within the next 100 years (Meinshausen et al., 2011) due to continued anthropogenic carbon emissions is generally predicted to increase net primary production (NPP) (Todd-Brown et al., 2014). Nevertheless such effects do not scale linearly with increases in atmospheric CO_2 because productivity is also strongly controlled by climate. Regions with drying climates are likely to present reduced net primary production in the next decades (IPCC, 2013). Although uncertainties exists in the magnitude of the changes (e.g. Campbell et al., 1991; Arnone and Körner, 1995; Gill et al., 2002), increases in net primary production are probably predominant and will simultaneously increase litter inputs to soils. Modification of atmospheric CO_2, of climate and of nutrient cycling may also modify the chemical composition of the litter (Cotrufo et al., 1999). Elevated CO_2 generally increases the lignin content and reduces the N concentration of plant tissues, although Norby et al., (2001) showed that this response depends on the experimental system used (open top chamber, free air CO_2 enrichment, etc.).

In past decades, the effects of litter addition on plant growth, soil C content and cycling, microbial biomass and priming of decomposition rates have been reported and confirmed (eg. Sulzman et al., 2005; Guenet et al., 2010; Jin et al., 2010; Chemidlin Prévost-Bouré et al., 2010; Sayer et al., 2011; Villalobos-Vega et al., 2011; Ma et al., 2012, Xiao et al., 2014). However, the impacts of litter addition on soil nutrients, plant growth and ecological C:N:P stoichiometry of plants, litter, soil and soil microbes remain highly uncertain. Moreover, climate change and increased atmospheric CO_2 would not only affect NPP but also environmental conditions for decomposers in soils (soil moisture, temperature), inducing modification of microbial community structure and activity (Singh et al., 2010). Associated to litter additions, these effects would disturb the C cycle in soil, affecting the net ecosystem exchange particularly in grasslands where the vast majority of the C stock is stored belowground, due to their high root:shoot biomass and productivity ratios (Mokany et al., 2006).

Grassland is one of the most important global terrestrial ecosystems, covering about 25% of the global terrestrial area and 40% of the land area in China (Kang et al., 2006). The semi-arid and temperate grasslands of northern China account for about 78% of the national grassland area, where the native vegetation is predominantly characterized by the abundance of grass species such as *Stipa* spp. and *Leymus chinensis* (Trin) Tzvel., with *Stipa krylovii* Roshev. being well-represented as one of the major steppe community types (Zhao et al., 2003). Plant recruitment, growth and nutrient cycling of the region often are limited by soil water, nitrogen and phosphorus, and the regional soil fertility and productivity are maintained by recycling of nutrients through plant litter decomposition as an essential mechanism (Liu et al., 2006) with little natural nitrogen deposition. A better understanding of the effects of litter addition on plant growth and ecological C:N:P stoichiometry of plants, litter, soil and soil microbes could help to reduce uncertainties in our predictions of C, N and P balance as well as cycling, and structure and function in grassland ecosystems under global climate change.

We conducted a field experiment in which we artificially added litter inputs to subsoils (i.e. 10-20 cm) under a *S. krylovii* steppe community in a temperate grassland of northern China to assess the effects on soil inorganic N and available P, plant growth, litter, C, N and P pools and the C:N:P stoichiometry of plant, litter, soil and soil microbes. The litter added was obtained under current atmospheric CO_2 concentration and therefore, the impact of elevated CO_2 on the litter stoichiometry (Cotrufo et al., 1999) was not represented in our case. The primary objectives of our study were to determine whether the studied grassland was resilient to litter addition or if litter additions would increase soil inorganic N and available P and thereby affect plant growth, litter, and the C, N, P pools and the C:N:P stoichiometry of plants, litter, soil and soil microbes. Here, we define the term resilience as the absence of a significant effect of litter additions on the measured variables (plant biomass, C:N:P ratio, etc.).

In a previous study (Xiao et al 2014), we observed that litter additions in the temperate grassland of northern China accelerate decomposition of soil organic matter through a priming effect. Priming is defined here as a modification of the soil organic matter decomposition rate induced by an input of litter and mediated by the altered activity of the microbial community (Blagodatskaya and Kuzyakov, 2008). The main objective of this study was to analyze how additional litter inputs and associated priming of soil organic matter decomposition alter nutrient availability and thereby plant stoichiometry and productivity. .

2. Materials and Methods

2.1 Study site

This study was conducted at a field site of the Duolun Restoration Ecology Experimentation and Demonstration Station of the Institute of Botany, the Chinese Academy of Sciences, located in south-eastern Inner Mongolia, northern China (Latitude 42°02′N, longitude 116°16′E, elevation 1350 m a.s.l.). The climate is temperate and semiarid with a dry spring and a wet summer. The long-term mean annual temperature at the site is 2.1°C, with monthly mean temperatures ranging from −17.5°C in January to 18.9°C in July. Mean annual precipitation is about 380mm, with 90% of the precipitation falling in the growing season between May and October.

Total precipitation was 196, 369, and 187 mm in 2009, 2010 and 2011, respectively, and the precipitation between May 1st and September 30th was 164, 314, and 159 mm in 2009, 2010 and 2011, respectively. Mean annual air temperature was 2.96, 2.43 and 2.11°C in 2009, 2010 and 2011, respectively (Fig. 1).

Soil type was classified as chestnut soil (Chinese classification) or Calcic Luvisols according to the FAO Classification (FAO, 1974). The soil characteristics were measured in the 0-30cm layer. Soils are composed of 63% sand, 20% silt, and 17% clay (Niu et al., 2010), with concentrations of soil organic C, N and P of 1.55%, 0.17% and 0.03%, respectively. The C:N:P ratio of soil is about 51.7:5.7:1. Compared to the global database of Xu et al., (2013), the soil of the study site is a nutrient rich soil. Mean bulk density is 1.31g cm^{-3}, and the soil pH is 7.7. The native vegetation is represented by typical steppe communities, where *Stipakrylovii* Roshev., a perennial bunchgrass, dominates. Other common species include *Leymus chinensis* (Trin) Tzvel, *Cleistogenes squarrosa* (Trin.) Keng, *Agropyroncristatum* (L.) Gaertner, *Artemisia frigid* Willd., *Potentillaacaulis* L., and *Carexduriuscula* CA Mey. Total vegetation cover was relatively sparse, ranging from 85 to

90%. Annual plant biomass production at the site was *c.*1000g (dry mass) m^{-2} $year^{-1}$ (Li et al., 2004).

2.2 Experimental design

On October 1st 2008, at the end of the growing season, we established twenty-five 2m ×1m treatment plots. Treatments involved the addition of fresh organic matter to the soil in the 10-20 cm soil layer. The applied fresh organic matter consisted of senescent above-ground tissues from an abundance-weighted mix of plant species occurring at the site. For this purpose, senescent plant biomass was harvested from an adjacent field, air-dried, fragmented, and passed through a sieve with a 2-mm mesh size. We used fragmented and sieved litter mainly to standardize the litter area available to microbes. The additions were equivalent to 0 (control treatment), 150, 300, 600 and 1200 g (dry mass) m^{-2}, with all arranged as a complete randomized-block design including five replications corresponding to litter input increases of 0, 15, 30, 60 and 120% respectively. It must be noted that net primary production is assumed to increase between 10 and 60% at the end of the 21st century (Arora and Boer, 2014; Todd-Brown et al., 2014), which means that the first three addition amounts are in the range of the expected increase. The plant litter used had a C concentration of 400.8 mg g^{-1} (standard error (SE) =1.3 mg g^{-1}, n=5), an N concentration of 9.72 mg g^{-1} (SE =0.04 mg g^{-1}, n=5), and a P concentration of 0.768 mg g^{-1} (SE =0.01 mg g^{-1}, n=5), corresponding to a C:N:P ratio of 521.9:12.6:1. Lignin concentration in litter was 190.9 mg g^{-1} (SE =0.9 mg g^{-1}, n=5).

Adding litter to the uppermost soil layers is impossible without drastically disturbing the soils. To minimize disturbance, we carefully removed the top 10-cm soil blocks, containing 60% of the root system (Zhou et al., 2007), with a sharp spade, keeping the soil blocks and vegetation as intact as possible. The soil underneath was loosened to a depth of 20 cm, and a predetermined quantity of plant litter was mixed homogeneously with the soil in the 10-20 cm layer. The surface soil blocks were then placed back into their original positions. Remaining fissures between the soil blocks were carefully filled with soil from the 0-10 cm soil layer and gently compacted by hand. To create consistent soil disturbance across treatments, the plots with zero litter addition were processed in the same manner as the plots that received plant litter. We did not add the litter at the soil surface, but inserted it in the soil to reduce export due to wind or rain and thereby better control the amount of litter added.

2.3 Field sampling and measurements

Field sampling of above-ground biomass, root biomass, litter and soils was conducted from August 1st to 3rd in 2009, 2010 and 2011. We choose this period because August is the peak of the growing season.

Above-ground biomass and litter were simultaneously sampled in each plot using a 1 m × 0.3 m quadrat. Live and dead above-ground biomass were measured by clip-harvesting and dead parts were combined with the litter. Below-ground biomass in the 0-20 cm soil layer was determined by soil coring to a depth of 20 cm using a cylindrical root sampler (8-cm inner diameter). Roots were manually removed from the soil samples. All samples of above-ground biomass, below-ground biomass and litter were oven-dried at 65°C to constant mass.

Soils were sampled in three different points of each plot within the 0-20cm soil layer with a soil sampler of 3 cm inner diameter. The samples were pooled and mixed to produce one composite sample. The fresh samples were sieved using a 2 mm sieve and visible plant tissues were removed. Then the samples were divided into two groups. The first group was immediately transported to the laboratory with a portable ice box and stored at 4 °C before microbial biomass analysis and the second group was dried by air in the shade. Additionally, soil bulk densities of the 0-20 cm soil layers of each plot were determined concurrently with soil sampling by a special coring device (volume = 100.0 ml).

In the lab, chemical analysis was performed on samples of above-ground biomass, below-ground biomass, litter and soil in the 0-20 cm soil layer for organic C and total N using an automatic elemental analyzer Vario EL III (Elementar Analysen systeme Comp., Hanau, Germany). Total P was determined by the H_2SO_4-$HClO_4$ fusion method (Sparks *et al.,* 1996). Soil microbial C and N biomass was measured by the fumigation-extraction method (Vance et al., 1987). Briefly, the fresh soil samples were adjusted to approximate 60 % of water holding capacity and then incubated for one week in the dark at 25 °C to reactivate soil microbes following the original procedure published by Vance et al. (1987). Next 20 g (dry weight equivalent) of fumigated with CH_3Cl for 24 hours and non-fumigated soil samples were both extracted with 0.5 M K_2SO_4. The extracts were filtered through 0.45-µm filters and the extractable C and N was analysed by dichromate and Kjeldahl digestion as described by Lovell et al. (1995). Soil microbial C and N biomass was calculated as the difference in extractable C and N contents between the fumigated and the unfumigated samples using conversion factors (k_{ec} and k_{en}) of 0.38 and 0.45 (Lovell et al., 1995), respectively. Mass ratios of C:N, C:P and N:P in plant, litter and soil samples and of C:N in soil microbial biomass were calculated and used to facilitate comparisons with previous studies (He et al., 2008). Additionally, 10 g dry soil samples in the 0-20 cm layer were extracted with 50 ml of 2 M KCl. Inorganic N (NH_4^+-N and NO_3^--N) of the filtered extracts of soils in the 0-20 cm soil layer were determined using a flow injection autoanalyzer (FIAstar 5000 Analyzer, Foss Tecator, Denmark). Available soil P in soils in the 0-20 cm soil layers was measured using the Olsen method (Olsen et al., 1954).

2.4 Statistical analysis

Data management and statistical analyses were performed using the SPSS software package (SPSS, Chicago, IL, USA). Two-way analysis of covariance (ANCOVA) was used to detect the effects of litter addition and year (sampling time) on soil inorganic N and available P, aboveground and belowground biomass, total biomass, litter, the C, N, P pools and C:N:P stoichiometry of plant, litter and soil, and C and N pools and C:N of soil microbial biomass. Multiple comparisons were also performed to permit separation of effect means using the Duncan test at a significance level of $P < 0.05$.

3. Results

3.1 Soil inorganic N and available P

Litter addition significantly enhanced soil inorganic N and available P in 2009, 2010 and 2011 (P < 0.05; Fig. 2), and there were significant differences in soil inorganic N and available P among different years (P < 0.01; Fig. 2). Nevertheless, these effects were mainly due to the highest input treatments. Indeed, for the years 2009 and 2011, only the highest litter inputs, corresponding to 1200 g DM m^{-2}, induced significant highest inorganic N and available P contents in soils. For the year 2010, the two highest litter inputs treatments induced significant increases of the inorganic N and available P contents in soils. There were no significant interactions between litter addition and year for soil inorganic N and available P (P > 0.05; Fig. 2) indicating that the effect of treatment was conservative over the year.

3.2 Plant biomass, carbon allocation and litter production

Significant effects of litter addition were observed for above-ground biomass and litter in 2009, 2010 and 2011 as well as for below-ground biomass and total biomass in 2010 and 2011 (P < 0.05; Fig. 3). In our study site, the belowground biomass was 6 times higher than the aboveground biomass. The ratio of belowground biomass to aboveground biomass in 2010 was significantly affected by litter inputs (P < 0.05; Fig. S1). However, it must be noted that the Duncan post hoc test showed that the effects described above are mainly due the highest input treatments, which is generally the sole treatment significantly different from the control when significant effects were detected by the two-ways ANCOVA. The highest litter addition increased biomass in all the compartments except for the belowground biomass and the total biomass in 2009. Furthermore, the highest litter addition treatment decreased the ratio of belowground biomass to aboveground biomass in 2010, while others treatments produced no effect. There were no significant interactions between litter addition and year on aboveground biomass, belowground biomass, total biomass, litter and ratio of belowground biomass to aboveground biomass ($P > 0.05$; Fig. 3 and 4). Therefore, for all these variables the treatment was conservative over the years.

3.3 C, N and P pools in plants, litter, soil and soil microbial biomass

Litter addition did not affect significantly the C, N and P pools of aboveground biomass and litter as well as soil C pools in 2009, 2010 and 2011 for all but the highest treatment. The C, N and P pools of belowground biomass in 2010 and 2011, and the C and N pools of soil microbial biomass were also not affected by litter addition except for the highest treatment ($P < 0.05$; Fig. 4). There were no significant differences in the C, N and P pools of aboveground biomass, the N and P pools of litter, the C pool of soil and the C and N pools of soil microbial biomass among different years for all treatment but the highest treatment ($P < 0.05$; Fig. 4). There were no significant interactions between litter addition and year, indicating a conservative treatment effect over the year on the C, N and P pools of aboveground biomass, belowground biomass, litter, soil and the C and N pools of soil microbial biomass ($P > 0.05$; Fig. 4). Even though C, N and P contents were significantly different for the highest treatment, for almost all the pools, they tended to increase when litter was added.

3.4 C, N and P stoichiometry in plant, litter, soil and soil microbe

Litter addition did not significantly modified C:N and C:P ratios of aboveground biomass and litter in 2010 and 2011 and the C:N ratio of soil microbial biomass in 2009, 2010 and 2011 for all but the highest treatment where a decrease was observed. The highest treatment significantly increased soil C:N and C:P ratios in 2009, 2010 and 2011 ($P < 0.05$; Fig. 5). But no effect was detected for the other treatments. Litter addition did not affect N:P of aboveground biomass, belowground biomass, litter and soil ($P > 0.05$; Fig. 5). There was significant difference in the C:N of aboveground biomass and soil microbial biomass among different years ($P < 0.05$; Fig. 5). There were no significant interactions between litter addition and year in effects on the C:N:P stoichiometry of aboveground biomass, belowground biomass, litter, soil and soil microbial biomass ($P > 0.05$; Fig. 5) indicating that the effect of treatment was conservative over the year.

4. Discussion

4.1 Effect on litter additions on plant growth

Plant growth is limited by the rate of resource supply, for example nutrients and water (Enquist et al., 2003). Furthermore, soil N and P are the main nutrient sources for plant growth (Elser et al., 2007; Vitousek et al., 2010; Alvarez-Clare et al., 2013; Fageria et al., 2013). The NPP in our study sites is expected to increase between 10 and 60% due to climate change and atmospheric CO_2 increase (Arora and Boer, 2014; Todd-Brown et al., 2014). Moreover, temperature is also expected to increase in China during the next decades (Piao et al., 2010), likely accelerating litter and soil organic matter decomposition and nutrient release.

Future predictions about the evolution of precipitation are still highly uncertain (Piao et al., 2010). The litter amendments we applied are substantial supply of nutrients, suggested to release nutrients during decomposition. Results show that availability of N and P were significantly modified only for the two highest inputs treatment. Additionally, high litter addition also greatly increased soil microbial biomass C and N, indicating that soil microbial biomass does plays an active role in nutrient transformation, conservation, and availability to plants (Wardle, 1992; Zaman et al., 1999; Tu et al., 2003). Notably, for more moderate litter additions, the observed effect on plant biomass was limited, suggesting that the plant did not benefit from these inputs. Litter addition significantly increased aboveground biomass in 2009, 2010 and 2011, and belowground biomass and total biomass in 2010 and 2011 only for the highest input level.

One plausible explanation for the lack of effect on plant biomass in spite of the important amount of organic nutrients added might be the imbalance between the C:N:P ratio of the litter added and the C:N:P ratio of the soil, the plants and the microbial community. For instance, the C:N and C:P ratios of the added litter were 41.3 and 521.9, respectively. Compared to the C:N ratio of microbial biomass around 12 and the C:N and C:P ratio of soil around 9 and 55, respectively, added litter decomposition might immobilize available mineral nutrients. As a consequence, the competition between plants and microorganisms for mineral N and available P would increase. Furthermore, a priming effect was observed during this experiment (Xiao et al., 2014) but the nutrients release associated might be not sufficient to increase the plant growth. Since plant biomass only responded to the highest litter treatments, it suggests that, in our case, microorganisms might be more efficient than plants in using nutrient resources and plant growth might be still nutrient limited. Nevertheless, soil microorganisms benefited only slightly from the litter inputs as suggested by the small observed increase in microbial biomass (Fig. 4m, 4n). Therefore, carbon use efficiency probably decreased when litter was added, increasing heterotrophic respiration. Such modification of the carbon use efficiency might be due to physiological modifications at the individual level, to a modification of the microbial community structure or to both mechanisms combined. With our data, we can’t estimate the carbon use efficiency at the individual level but based on the same experiment, Xiao et al., (2014) showed that the microbial community structure was different between treatments. It must be noted that the litter used here was harvested under natural conditions. Thus, the modifications of the litter chemical composition expected under climate change are not taken into account here. Litter C:N ratio may increase in the future (Norby et al., 2001) inducing a higher microbial N demand. Microorganisms may still decrease their carbon use efficiency as assumed here or may increase the N uptake. Since the microbial N uptake in our experiment only increased for very high and unrealistic litter inputs, we assume that, at our site, microorganisms will likely modify their carbon use efficiency in response to the modification of litter C:N ratio instead of competing with plants for N. Furthermore, when litter additions significantly affected plant biomass, aboveground biomass and belowground biomass were higher in 2010 than in 2009 and 2011. The years 2009 and 2011 were dry compared to the year 2010. Thus, a water stress may have limited the plant growth in 2009 and 2011. Moreover, soil moisture was likely higher in 2010. Such more favourable soil moisture conditions may have caused the higher soil nutrient availability via accelerated litter decomposition.

4.2 Biomass allocation

Biomass allocation is often affected by factors such as soil nutrient conditions and plant habitat (Vogt et al., 1983; Schmid, 2002; Mokany et al., 2006). In our study, high litter addition decreased the ratio of belowground biomass to aboveground biomass, and the decrease reached a significant level in 2010. The reason for this decrease may be that litter addition greatly increased soil organic N and available P and soil microbial biomass C and N, allowing the plant to invest more photosynthates in aboveground biomass in 2010 as already observed (Vogt et al., 1983; Schmid, 2002). Sims et al. (2012) found that adding nitrogen increased plant growth and allocated more biomass toward shoots than roots. The plastic response of increased allocation to shoots corresponds to theoretical predictions (Tilman, 1988). Such an increase in photosyntates concentration is also explained by the increase in C : N and C : P ratio in aboveground biomass and litter upon high litter addition, but not for belowground biomass.

4.3 Effect of litter addition on plant N:P ratio

Plant N and P are essential nutrients for primary producers and decomposers in terrestrial ecosystems, and N:P ratios of plant biomass or litter have been widely used as indicators of nutrient limitation for primary production (Koerselman and Meuleman, 1996; Tessier and

Raynal, 2003; Güsewell, 2004; Güsewell and Verhoeven, 2006). In our study, litter addition did not affect the N:P of aboveground biomass, belowground biomass and litter. Our results showed that the N:P of aboveground biomass (ranging from 13.3 to 13.9 under different litter additions) is higher than that of belowground biomass (ranging from 11.6 to12 under different litter additions). Similar results were observed in the study of Xu et al., (2010). The N:P of aboveground biomass and belowground biomass is lower than 14 on a community level, suggesting that our *S. krylovii* steppe community was N limited (Koerselman and Meuleman, 1996). Additionally, the N:P of litter ranged from 12.1 to 12.5 after different litter treatments and were much lower than 25 (the threshold between N and P limitation for graminoid leaf litter; Güsewell and Verhoeven 2006), also indicating that our *S. krylovii* steppe community is subject to N limitation. This result is consistent with the conclusion of Bai et al. (2012) who found that meadow steppe, typical steppe and desert steppe communities of temperate grasslands in northern China are N-limited systems.

5. Conclusion

In our study, litter addition significantly affected the stoichiometry of the systems only when it was quite high (twice the natural inputs). Previous modeling exercises have not predicted an increase of primary production sufficient to double litter inputs (Arora and Boer 2014; Todd-Brown et al., 2014). This suggests that, the grassland studied here is quite resilient in terms of stoichiometry. This resilience is the result of complex interactions between C and nutrients cycles as well as between plants and microbial biomass.

In conclusion, our results showed that very high litter addition increased soil inorganic N and plant available P, the C and N pools of soil microbial biomass, aboveground and belowground plant biomass. On the other hand, plausible additions according to the models predictions had no effect. This suggests that the expected increase of NPP may not have important consequences on the stoichiometric functioning for some particular ecosystems, such as grasslands in northern China. Nevertheless, it must be noted that climate change will also affect temperature and soil moisture, which will largely affect the response of ecosystems to modifications of NPP due to the atmospheric CO_2 increase.

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Figure captions

Fig. 1 Seasonal variations of weekly mean air temperature and weekly precipitation during the experimental period from 1 October 2008 to 31 September 2011.

Fig. 2 Soil inorganic N (a) and available P (b) in 2009, 2010 and 2011 under different amounts of litter addition. Vertical bars indicate one standard error about the mean (n=5). The soil inorganic N and the available P contents were different each year ($p<0.01$) but no interaction occurred between litter addition and year. Treatments with different letters are significantly different ($P < 0.05$) according to the Duncan test, small letters, capital letters and Greek letters correspond to the year 2009, 2010 and 2011, respectively. ‘ns’ implies that no significant differences were detected.

Fig. 3 Aboveground biomass (a), belowground biomass (b), total biomass (c) and litter (d) in 2009, 2010 and 2011 under different amounts of litter addition in a steppe community of northern China. Vertical bars indicate one standard error about the mean (n=5). A significant year effect ($p<0.05$) was detected by ANCOVA except for the below ground biomass. Interactions between litter addition and year were not significant. See Fig. 2 legend for letter signification.

Fig 4. The C, N, and P pools of plant, litter and soil and the C and N pools of soil microbial biomass in 2009, 2010 and 2011 under different amounts of litter addition in a steppe community of northern China. Vertical bars indicate one standard error about the mean (n=5). A significant year effect ($p<0.05$) on the C, N and P content was detected by ANCOVA except for the below ground biomass, the soil. No year effect for litter and soil microbial biomass C content was detected. Interactions between litter addition and year were not significant. See Fig. 2 legend for letter signification. Letters are only given where statistically significant differences are observed.

Fig. 5 The C:N, C:P and N:P ratio of plant, litter and soil and C:N ratio of soil microbial biomass in 2009, 2010 and 2011 under different amounts of litter addition in a steppe community of northern China. Vertical bars indicate one standard error about the mean (n=5). A significant year effect ($p<0.05$) on the C:N ratio of above ground biomass and soil microbial biomass was detected by ANCOVA. Interactions between litter addition and year were not significant. See Fig. 2 legend for letter signification. Letters are only given where statistically significant differences are observed.

646

647 Figure 1

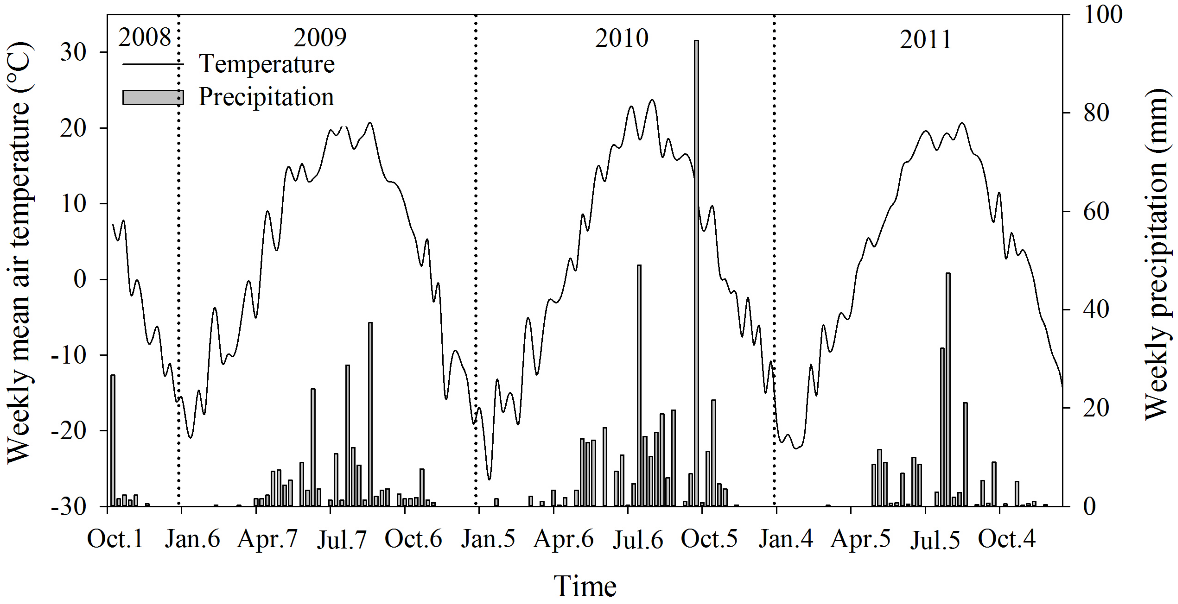

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Figure 2

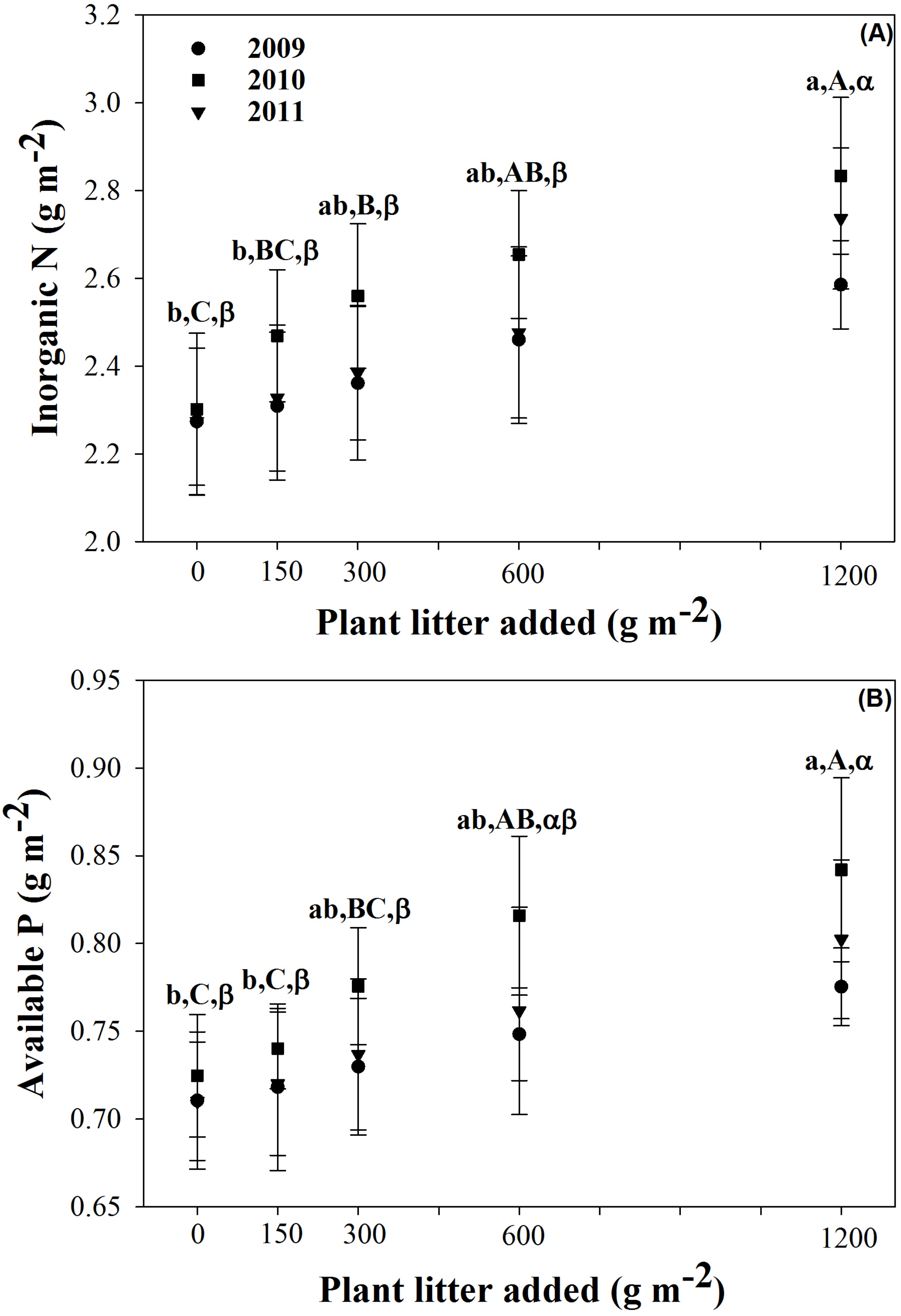

(A)
● 2009
■ 2010
▼ 2011
Inorganic N (g m^{-2})
3.2
3.0
2.8
2.6
2.4
2.2
2.0
b,C,β
b,BC,β
ab,B,β
ab,AB,β
a,A,α
0
150
300
600
1200
Plant litter added (g m^{-2})
(B)
Available P (g m^{-2})
0.95
0.90
0.85
0.80
0.75
0.70
0.65
b,C,β
b,C,β
ab,BC,β
ab,AB,αβ
a,A,α
0
150
300
600
1200
Plant litter added (g m^{-2})

Figure 3

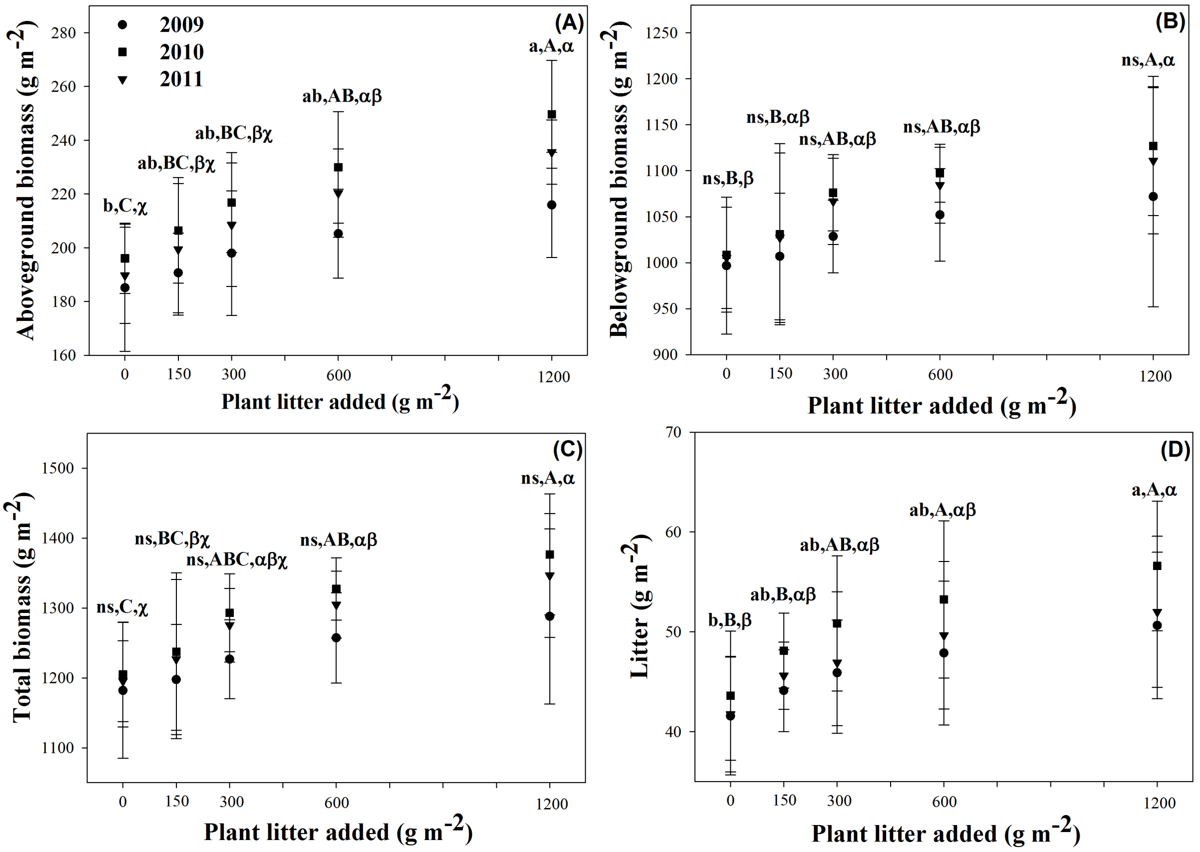

(A)
● 2009
■ 2010
▼ 2011
Aboveground biomass (g m-2)
160
180
200
220
240
260
280
b,C,χ
ab,BC,βχ
ab,BC,βχ
ab,AB,αβ
a,A,α
0
150
300
600
1200
Plant litter added (g m-2)
(B)
Belowground biomass (g m-2)
900
950
1000
1050
1100
1150
1200
1250
ns,B,β
ns,B,αβ
ns,AB,αβ
ns,AB,αβ
ns,A,α
0
150
300
600
1200
Plant litter added (g m-2)
(C)
Total biomass (g m-2)
1100
1200
1300
1400
1500
ns,C,χ
ns,BC,βχ
ns,ABC,αβχ
ns,AB,αβ
ns,A,α
0
150
300
600
1200
Plant litter added (g m-2)
(D)
Litter (g m-2)
40
50
60
70
b,B,β
ab,B,αβ
ab,AB,αβ
ab,A,αβ
a,A,α
0
150
300
600
1200
Plant litter added (g m-2)

Figure 4

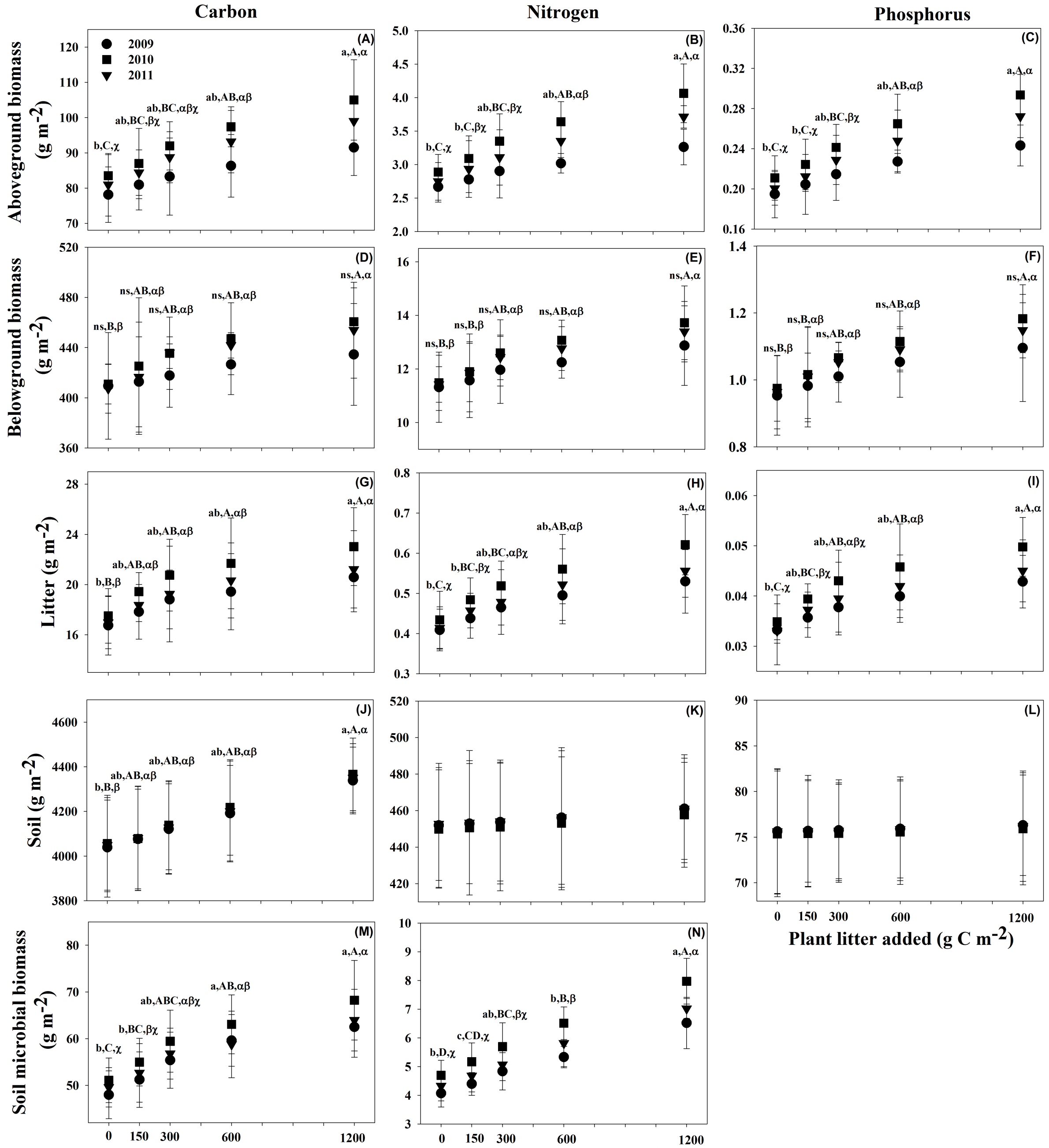
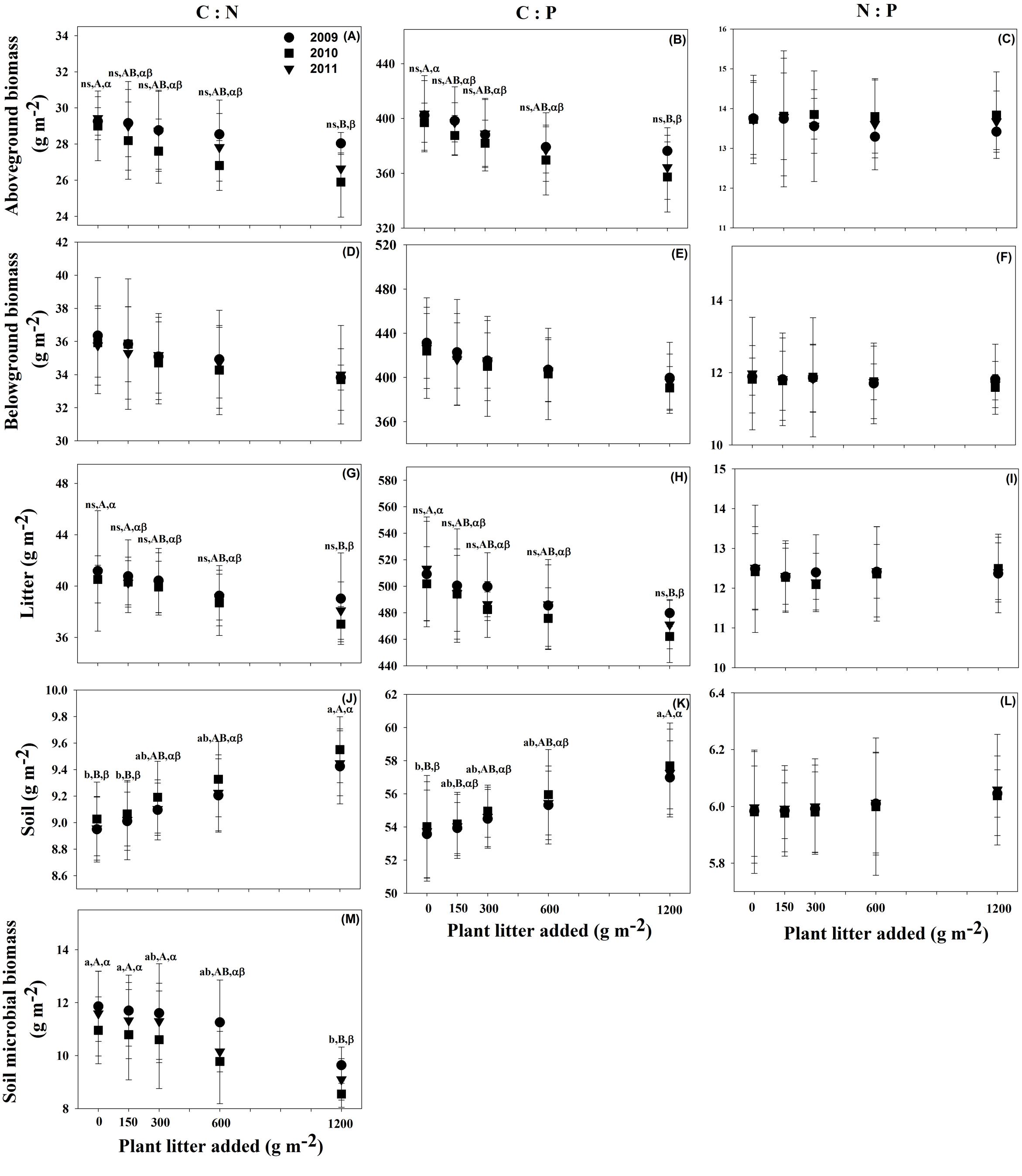

Carbon
Nitrogen
Phosphorus
Aboveground biomass (g m-2)
Belowground biomass (g m-2)
Litter (g m-2)
Soil (g m-2)
Soil microbial biomass (g m-2)
2009
2010
2011
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(I)
(J)
(K)
(L)
(M)
(N)
b,C,χ
ab,BC,βχ
ab,BC,αβχ
ab,AB,αβ
a,A,α
b,C,χ
b,C,βχ
ab,BC,βχ
ab,AB,αβ
a,A,α
b,C,χ
b,C,χ
ab,BC,βχ
ab,AB,αβ
a,A,α
ns,B,β
ns,AB,αβ
ns,AB,αβ
ns,AB,αβ
ns,A,α
ns,B,β
ns,B,β
ns,AB,αβ
ns,AB,αβ
ns,A,α
ns,B,β
ns,B,αβ
ns,AB,αβ
ns,AB,αβ
ns,A,α
b,B,β
ab,AB,αβ
ab,AB,αβ
ab,A,αβ
a,A,α
b,C,χ
b,BC,βχ
ab,BC,αβχ
ab,AB,αβ
a,A,α
b,C,χ
ab,BC,βχ
ab,AB,αβχ
ab,AB,αβ
a,A,α
b,B,β
ab,AB,αβ
ab,AB,αβ
ab,AB,αβ
a,A,α
b,C,χ
b,BC,βχ
ab,ABC,αβχ
a,AB,αβ
a,A,α
b,D,χ
c,CD,χ
ab,BC,βχ
b,B,β
a,A,α
0
150
300
600
1200
Plant litter added (g m-2)
Plant litter added (g C m-2)

Figure 5


C : N
C : P
N : P
2009
2010
2011
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(I)
(J)
(K)
(L)
(M)
Aboveground biomass (g m-2)
Belowground biomass (g m-2)
Litter (g m-2)
Soil (g m-2)
Soil microbial biomass (g m-2)
Plant litter added (g m-2)
0
150
300
600
1200
ns,A,α
ns,AB,αβ
ns,AB,αβ
ns,AB,αβ
ns,B,β
ns,A,α
ns,AB,αβ
ns,AB,αβ
ns,AB,αβ
ns,B,β
ns,A,α
ns,A,αβ
ns,AB,αβ
ns,AB,αβ
ns,B,β
ns,A,α
ns,AB,αβ
ns,AB,αβ
ns,AB,αβ
ns,B,β
b,B,β
b,B,β
ab,AB,αβ
ab,AB,αβ
a,A,α
b,B,β
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ab,AB,αβ
ab,AB,αβ
a,A,α
a,A,α
a,A,α
ab,A,α
ab,AB,αβ
b,B,β
24
26
28
30
32
34
320
360
400
440
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42
360
400
440
480
520
10
12
14
36
40
44
48
440
460
480
500
520
540
560
580
10
11
12
13
14
15
8.6
8.8
9.0
9.2
9.4
9.6
9.8
10.0
50
52
54
56
58
60
62
5.8
6.0
6.2
6.4
8
10
12
14