Nitrogen addition and mowing affect microbial nitrogen transformations in a C4 grassland in northern China

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Summary
Microbial nitrogen (N) transformations play a key role in regulating N cycling in grassland ecosystems. However, there is still little information on how management of semi-arid grassland such as mowing and/or N fertilizer application affects microbial activity and N transformations. In a field experiment in northern China, N was added at a rate of 10 g N m$^{-2}$ year$^{-1}$ as NH$_4$NO$_3$ to mown and unmown plots ($4 \times 4$ m$^2$) and in situ rates of net ammonification ($R_{amn}$), nitrification ($R_{ni}$) and mineralization ($R_{min}$) were followed at monthly intervals for the vegetation growth periods in the years 2006–2009. In addition, we also measured soil microbial biomass carbon (MBC) and nitrogen (MBN), microbial respiration (MR) and peak above-ground biomass in August of each measurement year. Driven by the pronounced inter-annual variability of rainfall, all the properties investigated varied markedly across years. Nevertheless, we were able to demonstrate that over the 4 years N addition significantly stimulated $R_{ni}$, $R_{min}$ and MBN, on average, by 288, 149 and 11.6%, respectively. However, N addition decreased MBC significantly as well as the ratio of MBC:MBN by, on average, 10 and 23%, respectively, whereas an effect of N addition on MR could not be demonstrated. Mowing decreased MBN, MR and $q$CO$_2$ significantly by 9, 28 and 24%, respectively, but no effects were found on microbial net N transformation rates and MBC. N addition and mowing interactively affected $R_{amn}$ and $R_{min}$, and MBN, MBC:MBN. In summary, our results indicate a positive effect of N addition but a negative effect of mowing on microbial N transformation in this C4 grassland in northern China.

Introduction
During the last century, accelerating industrialization and agricultural activity increased nitrogen (N) deposition dramatically in most parts of the world (Galloway et al., 2008). In northern China, rates of atmospheric N deposition are likely to increase by 2–5 g m$^{-2}$ year$^{-1}$ above pre-industrial rates by 2050 (Galloway et al., 2004). For the terrestrial biosphere, the enrichment of N by direct fertilizer application and atmospheric N deposition has been shown to increase plant growth, net primary productivity and biomass accumulation at a global scale (Elser et al., 2007; LeBauer & Treseder, 2008; Xia & Wan, 2008) and to increase the mobility of N (Vitousek et al., 1997). It may also alter the rates and the pathways of soil–plant N cycling and associated hydrological and gaseous losses (Aber et al., 1989).

The extent to which increased N inputs will drive changes in plant productivity and species composition over the next century will, however, depend on N retention in ecosystems (Zavaleta et al., 2003). Nitrogen retention and reduction of N losses may also be linked to N fertilizer addition, because this increases plant productivity and extends the time over which roots remain active. However, the addition of inorganic N can also inhibit the decomposition of soil organic matter because inorganic N is incorporated into recalcitrant compounds that are formed during lignin degradation (Berg & Matzner, 1997). On the other hand, mowing accelerates the N cycle (Güsewell et al., 2005) and promotes increased above- and below-ground plant growth and root exudation (Leriche et al., 2001) and this may increase the risk of N losses. Other effects of hay harvesting or mowing are linked to changes in the above-ground community structure (Knapp et al., 2002), which, in turn, affect N uptake patterns by the vegetation. Above-ground biomass removal can also reduce C inputs to the soil significantly,
resulting in substrate limitation to microbes (Wan & Luo, 2003). Biomass removal may therefore mask the N fertilizer effects on soil microbial properties. In addition, reduced vegetation cover after hay harvesting or mowing may change the soil boundary layer near the surface, increase the energy absorbed and emitted by the soil, and amplify the diurnal soil temperature range. Finally, plant removal can increase evaporation while decreasing transpiration, resulting in an unpredictable net effect on soil moisture. Therefore, N fertilizer addition and changes in land management may interactively affect soil microbes under future climatic conditions.

Agricultural restructuring and conservation priorities have led to the abandonment of farmland in China and elsewhere. Thus, during the last decade, restoration of croplands to grasslands has been observed across grassland systems of Inner Mongolia in northern China, with grasslands or steppe representing the typical climax vegetation type in this arid and semi-arid environment (Christensen et al., 2004). Mowing of grassland is an important management practice in the area. Nitrogen availability and microbial N transformation processes are key characteristics for the evaluation of this management practice because they greatly affect steppe productivity. However, few studies have evaluated their relative importance and interaction in old fields in China.

This paper complements our previous work at the same experimental site (Wang et al., 2011), which addressed questions on nutrient availability and clipping for three different plant communities (grass, grass-herb and herb dominated) separately. In contrast, the current study is a 4-year study, focusing on grass dominated patches. The specific objectives of this study were to evaluate the individual and combined effects of mowing and N addition on (i) in situ net N mineralization and (ii) microbial biomass and activities. Because annual rainfall as well as that occurring during the growth period is highly variable we also hypothesized that (iii) effects of mowing and N fertilization are more pronounced in years with greater annual precipitation than in dry years.

Materials and methods

Site description

The experimental site is located in Dunlun county (42°27’N and 116°40’E), a semi-arid area in Inner Mongolia, north China. The mean annual precipitation in this region is 382.2 mm. The average annual temperature is 2.1°C, with monthly mean temperatures ranging from −17.6°C in January to 19.0°C in July, according to a long-term observation period (1953–2007) at Duolun Restoration Ecology Experimentation and Demonstration Station (Wang et al., 2011). The soil at the sites is a chestnut soil (Chinese classification; Wang et al., 2011) or Calcic Luvisol (FAO; IUSS, 2007). Soil and plant characteristics were measured before start of the experiment in August of 2005 and reported by Wang et al. (2011). In the study area, large parts of the natural steppe were converted to cropland in the 1960s. As a restoration measure, about 80% cropland was abandoned in 1995 and either remained without any further management or has been used for hay production or grazing.

Experimental design

Twenty-four 4 × 4 m² plots were established in 2005. Four different treatments organized in a randomized block design were investigated: (i) control, (ii) addition of N fertilizer, (iii) mowing of above-ground biomass and (iv) a combination of mowing and N fertilizer addition. Mowing was carried out at the end of August in 2006, 2007, 2008 and 2009, and removed all above-ground biomass above 3 cm. Fertilizer as NH₄NO₃ was added before the rains in mid-July of 2006–2009 at a rate of 10 g N m⁻² to the fertilizer treatments. Our previous paper reported the data from the growing season (from May to October) in 2008 (Wang et al., 2011).

The top (0–10 cm) layer of mineral soil was sampled from all 24 plots in June, July and August to determine net N mineralization, nitrification and ammonification rates for each year. Potential microbial biomass carbon (MBC) and N (MBN), as well as microbial respiration (MR), were measured once a year only in the middle of August (see later) when the most vigorous microbial activity occurred in this area (Liu et al., 2009). Soil temperature and soil moisture were measured at a depth of 10 cm (Wang et al., 2011).

Sampling and measurements

The buried soil core technique was used to measure in situ net N turnover during the growing season for each year. The detailed techniques were described by Wang et al. (2011). Above- and below-ground biomass was measured before the mowing treatment each year. Two 2 × 0.5 m² subplots in each plot were clipped at the soil surface to measure above-ground biomass in the middle of August during 2006–2009. After the harvest, two soil cores with a diameter of 6.5 cm at 0-15-cm depth were sampled in each plot to determine root biomass. Root samples were placed in a cooler and transported to the laboratory. In the laboratory, the cores were soaked in deionized water and residual soil was washed from roots over 0.5-mm sieve. Plant and root materials were oven-dried at 65°C for 48 hour and weighed. Inorganic N was determined in each sieved soil sample after extraction of 10 g dry soil with 50 ml 2 mol l⁻¹ KCl solution (see Wang et al., 2011 for details). The process and methods of potential microbial biomass and respiration measurements were as described by Wang et al. (2011) and Vance et al. (1987). All results were expressed on an oven-dried soil basis (105°C, 24 hour). Soil organic carbon (C) was measured with the potassium dichromate-vitriol oxidation method and soil total N was measured by Kjeldahl digestion (Cabrera & Beare, 1993).

Statistical analysis

The seasonal mean values used in this study were calculated from the monthly mean values, which were first averaged from all measurements in the same month. A three-way ANOVA was used to examine the effects of year, mowing, N addition and their possible interactions, on microbial N transformation, biomass and
Results

Effects of mowing and N addition on soil temperature and moisture

Probably because of limited measurement of soil temperature and soil moisture over time, significant effects of mowing and N fertilization were only observed at some measuring times. There was a trend for mowing to increase soil temperature at 10-cm depth by 0.8°C (Figure 1) over 4 years. There were strong inter-annual fluctuations of rainfall during the growing season (May–October), with 387.2, 185.6, 318.0 and 176.1 mm in 2006, 2007, 2008 and 2009. CC, control; CN, N addition; FC, mowing; FN, mowing plus N addition.
and 2009, resulting in marked effects on annual average soil moisture contents; this was most pronounced in the N addition plots ($P < 0.05$). Soil temperature also varied markedly across the 4 years ($P < 0.001$); this was most pronounced for the mown plots ($P < 0.001$).

**Soil and plant characteristics**

Mowing and N addition + mowing significantly increased soil total organic C content by 39 and 50%, respectively, after 4 years (Table 1; $P < 0.05$). However, there were no significant effects found in total N content among the mowing, N addition and mowing + N addition treatments (Table 1; $P > 0.05$). Increased total soil organic carbon therefore resulted in the C:N ratio increasing significantly after mowing (Table 1; $P < 0.05$). Nitrogen addition decreased pH values significantly by about 0.6 units ($P < 0.05$), but increased above-ground net primary production (ANPP, $P < 0.001$) and below-ground net primary production (BNPP, $P < 0.01$) to around 90 and 400 g m$^{-2}$, respectively. N addition + mowing significantly increased ANPP ($P < 0.05$) but no effects were found for BNPP (Table 1; $P > 0.05$).

**Above-ground biomass (including standing litter)**

Nitrogen addition increased ANPP (314 ± 25 g m$^{-2}$) significantly by 48%, but there was not an effect of mowing on ANPP in either the non-fertilized or fertilized plots over the 4 years (Table 1, Figure 2a; $P < 0.01$). Nitrogen addition had no effect on standing litter biomass, while mowing and N + mowing significantly reduced standing litter over the 4 years (2006–2009) (Figure 2b; $P < 0.01$).

Nitrogen addition significantly increased the total above-ground biomass (ANPP + standing litter, 685 ± 56 g m$^{-2}$) by 46.3% only in 2008, which was the second wettest growing season after 2006 (Figure 2c; $P < 0.05$). In the other 3 years, N addition had no effects on total above-ground biomass ($P > 0.05$), but significantly increased ANPP in 2006, 2008 and 2009 ($P < 0.01$). Mowing significantly reduced total above-ground biomass by 65.8 and 75.9% in 2007 and 2008, respectively ($P < 0.05$). In particular, ANPP was significantly larger in 2008 after N addition treatment than in the other 3 years ($P > 0.05$). However, mowing had no effects on ANPP in rainy (2006 and 2008) or drought (2007) years ($P > 0.05$).

Over the 4 years, mowing decreased standing litter significantly by 257% ($P < 0.001$), whereas N addition had no effect ($P > 0.05$). Total above-ground biomass (green biomass + litter standing, 270 ± 33 g m$^{-2}$) was significantly decreased by 64% after mowing ($P < 0.001$), and increased by 48% with N addition averaged over 4 years ($P < 0.001$).

**Soil ammonification, nitrification and net N mineralization rates**

There was strong intra- and inter-annual variability in net N transformation (ammonification rate, nitrification rate and net N mineralization rate), which could be inferred from the significant

![Figure 2](image-url)
Nitrogen turnover in semi-arid grassland

Time (rMANOVA) and year effects (three-way ANOVA; Table 2, Figure 3) that we recorded. However, significant differences among treatments with regard to ammonification, nitrification and net N mineralization were found only in 2006 and 2008 (Figure 3; P > 0.05) and the mowing + N addition and N addition treatments for ammonification rate in 2007.

Over the entire observation period of 4 years, mowing increased the nitrification rate significantly by 106% (P < 0.001). Also, N addition stimulated rates of nitrification and net N mineralization by 286% (P < 0.001) and 149% (P < 0.01), respectively (Table 2). The mowing and N addition interaction affected the ammonification and net N mineralization rates (P < 0.05). The year × mowing and year × N addition had interactive effects on the nitrification (P < 0.001) and net N mineralization rates (P < 0.01).

There were also three-way interactive effects (year × mowing × N addition) on the ammonification and net N mineralization rates (P < 0.01).

Soil microbial biomass and respiration

In 3 of the 4 years no significant differences in MBC were found among the four treatments (Figure 4; P > 0.05). Only in 2006 (the first year of N fertilizer addition and also the year with the largest precipitation rate during the growth period) mowing + N addition decreased MBC significantly by 21% (P < 0.01). Fertilizer N tended to increase MBN, specifically in the non-mowed plot, with differences being mostly significant at P < 0.05. However, a significant (P < 0.05) reduction in the MBC:MBN ratio was found only in the first year of N fertilizer application (2006) (Figure 4).

Mowing + N addition reduced microbial respiration (MR) significantly in 3 of the 4 years (range 43.8–89.9%), but not in the driest year (2009). In the first treatment year (2006) mowing decreased MR by 93.6% (P < 0.01) and qCO₂ by 85.7% (Figure 5, P < 0.01), but in the following years these effects diminished.

Driven by the large variability amounts of rainfall in the growing period across the year, substantial inter-annual variability in all of the microbial variables (Table 3, P < 0.001) was observed. When averaged across the 4 years, N addition significantly decreased MBC by 12% (P < 0.05) and increased MBN by 12% (P < 0.05). Mowing marginally decreased MBC and MBN, both by 10% (P = 0.05), and decreased MR and qCO₂ significantly by 28% (P < 0.001) and 23% (P < 0.01), respectively. The interactive effects of N addition and mowing were evident only for MBN (P < 0.01) and the MBC:MBN ratio (P < 0.05) (Table 3), but not for MR and qCO₂.

Discussion

Effects of nitrogen addition

Although the effect of N additions on soil microbial activity and ecosystem N cycling is a major focus of ecosystem research (Hassink, 1992; Fisk & Fahey, 2001; Maly & Sarapata, 2002; Chu & Lin, 2007; Treseder, 2008; Wang et al., 2011), information on the effects of N addition on N cycling in semi-arid grassland ecosystems remains limited, especially for systems in central and eastern Asia. During the past decade, restoration of croplands to grasslands has become an important management practice in northern China. Use of natural grasslands for agriculture can result in large losses of soil organic matter and nitrogen. Thus abandoned cropland provides not only a valuable case study for N cycling and microbial activity but an important challenge for the practice of ecological restoration now and in the future. Dijkstra et al. (2005) found for a grassland ecosystem in central USA that N fertilizer stimulated net N mineralization. They argued that the stimulation resulted from either faster decomposition or reduced N immobilization by litter with larger N concentrations when the soil was wetter. In our study in old fields of northern China we also found an overall stimulation of net microbial N turnover rates by N additions over 4 years. Furthermore, microbial biomass N was also increased for the N-fertilized treatments during that time. Both findings indicate that microbial N turnover and the microbial community is severely N limited at our sites, which agrees with findings for tall-grass prairie systems in North America (Garcia & Rice, 1994). The overall trend of stimulation of microbial N cycling at our site across years is remarkable because the amounts of precipitation in the growing season were very variable across the four study years between 2006 and 2009. This is in contrast to results for the year 2008 as reported by Wang et al. (2011) for the same site. However, the data of 2008 were collected from three different patches, including herb or grass-dominated patches and mixed herb-grass patches. Even though data for 2008 still do not reveal a stimulating effect of N additions on microbial biomass N, all other years show such a stimulation effect. Even in dry years such as 2009, when the annual precipitation (176.1 mm) was only approximately 50% of the 55-year mean precipitation (348 mm), a stimulating effect of N addition on microbial N turnover and MBN could be demonstrated. This shows that multi-year experiments are needed to evaluate microbial community responses to changes in nitrogen availability.

So far the effect of N additions on potential changes in microbial activity has been largely neglected (Treseder, 2008). This might be because N addition negatively affected microbial growth in several field and laboratory studies (Soderstrom et al., 1983; Nohrstedt et al., 1989). Results from our experiment showed that the microbial biomass and respiration had changed significantly after 4 years of N fertilizer addition to mown fields. In addition, N fertilizer without mowing significantly decreased potential MBC and MR. Reductions in MBC and MR, plus our findings that at treatment sites with N addition MBN was significantly increased while the MBC:MBN ratio was significantly reduced, may indicate a shift in the microbial community structure, although this was not studied here. In view of the narrowing of the MBC:MBN ratio, which was most pronounced in the first year of fertilizer input, we suggest that at our site the soil microbial community shifted in response to N additions from being fungi- to bacteria-dominated. This interpretation is in agreement with findings for temperate grasslands and pastures where reductions in abuscular mycorrhizal fungi
Table 2 Summary table for repeated ANOVA of soil temperature (ST) and soil moisture (SM), ammonification rate ($R_{ammon}$), nitrification rate ($R_{nit}$), and net N mineralization rate ($R_{\text{min}}$)

| Source of variation | Degrees of freedom | Sum of squares | Mean square | F      | P      |
|---------------------|--------------------|----------------|-------------|--------|--------|
| **Soil temperature** |                    |                |             |        |        |
| Year                | 3                  | 265.312        | 88.437      | 89.51  | < 0.0001 |
| Clip                | 1                  | 20.203         | 20.203      | 20.45  | < 0.0001 |
| Year × clip         | 3                  | 5.777          | 1.926       | 1.98   | 0.1284 |
| Fertilizer          | 1                  | 0.863          | 0.863       | 0.87   | 0.3529 |
| Year × fertilizer   | 3                  | 0.1831         | 0.0061      | 0.06   | 0.9798 |
| Clip × fertilizer   | 1                  | 0.38           | 0.38        | 0.38   | 0.5369 |
| Y × C × F           | 3                  | 0.8702         | 0.2901      | 0.29   | 0.8299 |
| Residuals           | 80                 | 79.04          | 0.988       |        |        |
| Total               | 95                 | 372.63         |             |        |        |
| **Soil moisture**   |                    |                |             |        |        |
| Year                | 3                  | 1230.03        | 410.01      | 171.45 | < 0.0001 |
| Clip                | 1                  | 3.33           | 3.33        | 1.39   | 0.2415 |
| Year × clip         | 3                  | 3.657          | 1.219       | 0.51   | 0.6767 |
| Fertilizer          | 1                  | 9.601          | 9.601       | 4.01   | 0.0485 |
| Year × fertilizer   | 3                  | 14.2749        | 4.7583      | 1.99   | 0.1221 |
| Clip × fertilizer   | 1                  | 35.4051        | 35.4051     | 14.81  | 0.0002 |
| Y × C × F           | 3                  | 93.7925        | 31.2642     | 13.07  | < 0.0001 |
| Residuals           | 80                 | 191.312        | 2.3914      |        |        |
| Total               | 95                 | 1581.4         |             |        |        |
| **Ammonification rate** |                 |                |             |        |        |
| Year                | 3                  | 3.2067         | 1.0689      | 32.12  | < 0.0001 |
| Clip                | 1                  | 0.0104         | 0.0104      | 0.31   | 0.5778 |
| Year × clip         | 3                  | 0.1498         | 0.0499      | 1.5    | 0.2206 |
| Fertilizer          | 1                  | 0.03654        | 0.03654     | 1.1    | 0.2978 |
| Year × fertilizer   | 3                  | 0.2334         | 0.0778      | 2.34   | 0.0798 |
| Clip × fertilizer   | 1                  | 0.1456         | 0.1456      | 4.38   | 0.0396 |
| Y × C × F           | 3                  | 0.5891         | 0.1964      | 5.9    | 0.0011 |
| Residuals           | 80                 | 2.662          | 0.033       |        |        |
| Total               | 95                 | 7.034          |             |        |        |
| **Nitrification rate** |                 |                |             |        |        |
| Year                | 3                  | 0.732          | 0.244       | 20.4   | < 0.0001 |
| Clip                | 1                  | 0.1017         | 0.1017      | 8.5    | 0.0046 |
| Year × clip         | 3                  | 0.2874         | 0.0958      | 8.01   | < 0.0001 |
| Fertilizer          | 1                  | 0.2517         | 0.2517      | 21.05  | < 0.0001 |
| Year × fertilizer   | 3                  | 0.4134         | 0.1378      | 11.52  | < 0.0001 |
| Clip × fertilizer   | 1                  | 0.0226         | 0.0226      | 1.89   | 0.1728 |
| Y × C × F           | 3                  | 0.0394         | 0.0131      | 1.1    | 0.3546 |
| Residuals           | 80                 | 0.9568         | 0.012       |        |        |
| Total               | 95                 | 2.8051         |             |        |        |
| **Mineralization rate** |              |                |             |        |        |
| Year                | 3                  | 6.0377         | 2.0125      | 28     | < 0.0001 |
| Clip                | 1                  | 0.2111         | 0.2111      | 2.94   | 0.0904 |
| Year × clip         | 3                  | 0.8403         | 0.2801      | 3.9    | 0.0118 |
| Fertilizer          | 1                  | 0.673          | 0.673       | 9.36   | 0.003  |
| Year × fertilizer   | 3                  | 0.9019         | 0.3006      | 4.18   | 0.0084 |
| Clip × fertilizer   | 1                  | 0.2797         | 0.2797      | 3.89   | 0.052  |
| Y × C × F           | 3                  | 0.8952         | 0.2984      | 4.15   | 0.0087 |
| Residuals           | 80                 | 5.7507         | 0.0718      |        |        |
| Total               | 95                 | 15.5897        |             |        |        |
Nitrogen turnover in semi-arid grassland

Figure 3 Seasonal dynamics of ammonification (Ramm), nitrification (Rnit) and net N mineralization (Rmin) rates (mean ± SE) from 2006 to 2008. CC, control; CN, N addition; FC, mowing; FN, mowing plus N addition.

(Bradley et al., 2006) or decreased fungal fatty acid methyl ester abundance (de Vries et al., 2007; Rousk et al., 2011) were found in response to N additions. In contrast to our study, Pietikäinen et al. (2005) found that N addition did not affect microbial N but considerably increased plant N in a sub-arctic meadow. In our study we also found that N addition increased plant productivity by more than 30% in the second and third years of the experiment (Figure 2). Thus, the stimulation of soil microbial N turnover following N fertilization is probably not only a response to increased soil N availability but also a feedback to N-induced increases in plant growth. This interpretation is in line with the work of Hu et al. (2001), who found for grassland ecosystems that stimulation of plant growth in response to elevated CO2 exacerbated nitrogen constraints on microbes so that N additions are required to avoid a reduction in microbial decomposition.

Mowing effects

Above-ground biomass removal can reduce C inputs to soil significantly and lead to significant N loss, resulting in substrate limitation to microbes (Wan & Luo, 2003). However, against our expectation of negative impacts of mowing and litter removal on microbial N turnover, we found that mowing increased nitrification rates and did not affect the average ammonification and net N mineralization rates throughout the study period of 4 years. Mowing may result in increased soil temperature and, at humid temperate grassland sites, soil moisture values (Bardgett et al., 1998; Tix et al., 2006), but these effects could not be demonstrated in our study because we had too few measurements. Another mechanism related to how plant removal may interact with soil N cycling was revealed by Zak et al. (1994). These authors demonstrated that the removal of plant material with a large C:N ratio facilitated rapid N cycling by limiting carbon input to the soil, thus maintaining rapid N turnover in the microbial community in an old field in Minnesota, USA.

Our results showed that over a 4-year period mowing decreased MBC and MBN on average by 9.8 and 10.2% (Table 3). However, mowing only decreased MBN during the whole growing season in 2008 (Wang et al., 2011). These findings are in contrast to a study for an upland grassland in England where mowing enhanced soil microbial biomass (Bardgett et al., 1998). However, our observation of a decline of MBC and MBN after mowing is further backed up by the observed significant reduction in microbial respiration (28% less than the control) and a decrease of the qCO2 by 24%. This indicates that responses of microbial C and N dynamics to mowing differ markedly between moist and semi-arid temperate grasslands. Also in other grassland ecosystems with a vegetation period restricted by environmental constraints, such as a sub-arctic meadow at Kilpisjärvi, Finnish Lapland, it was found that mowing reduced microbial respiration and the metabolic quotient of the microbial community in both fertilized and unfertilized treatments (Stark & Kytöviita, 2006). A smaller metabolic quotient indicates a more efficient use of substrates by microorganisms, where a greater fraction of substrate C is incorporated into microbial biomass and less C per unit biomass is lost through respiration (Ilstedt et al., 2003). The reduction of microbial availability of C that is mediated by mowing is likely to result from a decreased plant C flow to soil in

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Interactive effects of N addition and mowing

Land management practices such as hay harvesting may significantly influence the direct impacts of N addition on the soil environment and on soil microbial N transformation responses to N addition (Shaver et al., 2000). Above-ground biomass removal by mowing may mask the N fertilizer effects on soil microbial communities by reducing plant litter input and root exudation and thus reducing soil microbial biomass and activities. In our study a significant effect of N fertilizer on microbial respiration could not be demonstrated either for mowed or for uncut plots. Nitrogen fertilizer applied to old fields did effectively promote plant growth. However, the removal of up to 90% of plant litter by mowing probably resulted in a depletion of labile C sources in the topsoil and, together with increased N uptake by the plants as a response to mowing, soil microbes may have become C as well as N limited. This explains our finding that microbial respiration did not increase. This interpretation is in line with the studies by Johnson et al. (2000) and Stark & Kyttöviita (2006), who found that fertilizer increased in situ soil respiration (plant plus microbial respiration) but did not affect the microbial respiration measured in the laboratory from sieved soil samples. Results from our experiments indicate that 4 years of continuous N fertilizer application without mowing led to significant changes in microbial N transformation in the old field ecosystem we studied, resulting in an increase in rates of net N mineralization. However, mowing significantly masked this N fertilizer effect on soil microbial turnover rates.

Conclusions

In conclusion, the experiment that we conducted in an old field of northern China showed that over a 4-year period (2006–2009) mowing and N addition simultaneously increased N transformation. However, mowing and N addition showed strong interactive effects: while mowing decreased microbial biomass N (MBN), MBN was increased by N fertilizer addition. This indicates that expected further increases in atmospheric nitrogen deposition to the study region or possibly large-scale N fertilization for increasing ecosystem productivity will accelerate N cycling, thereby increasing the risk of N losses to the environment. Adaptation of grazing or mowing regimes are, according to our findings, possible options to keep N...
Table 3 Summary table for repeated ANOVA of microbial biomass C (MBC), microbial biomass N (MBN), microbial respiration (MR) and $\varphi$CO$_2$

| Source of variation | Degrees of freedom | Sum of squares | Mean square | F      | P      |
|---------------------|--------------------|---------------|-------------|--------|--------|
| **MBC**             |                    |               |             |        |        |
| Year                | 3                  | 6888.080      | 229360      | 55.93  | < 0.0001 |
| Clip                | 1                  | 15988.52      | 15989       | 3.9    | 0.0518 |
| Year × clip         | 3                  | 2878.062      | 959.35      | 0.23   | 0.8725 |
| Fertilizer          | 1                  | 22019.32      | 22019       | 5.37   | 0.0231 |
| Year × fertilizer   | 3                  | 1947.46       | 615.82      | 0.15   | 0.9293 |
| Clip × fertilizer   | 1                  | 464.2441      | 464.24      | 0.11   | 0.7374 |
| Y × C × F           | 3                  | 15254.06      | 5084.7      | 1.24   | 0.3008 |
| Residuals           | 80                 | 328072.3      | 4100.9      |        |        |
| Total               | 95                 | 1074605       |             |        |        |
| **MBN**             |                    |               |             |        |        |
| Year                | 3                  | 26439.47      | 8813.2      | 17.12  | < 0.0001 |
| Clip                | 1                  | 2042.784      | 2042.8      | 3.97   | 0.0498 |
| Year × clip         | 3                  | 930.001       | 310         | 0.6    | 0.6154 |
| Fertilizer          | 1                  | 2607.08       | 2607.1      | 5.07   | 0.0272 |
| Year × fertilizer   | 3                  | 1309.246      | 436.42      | 0.85   | 0.4717 |
| Clip × fertilizer   | 1                  | 4226.229      | 4226.2      | 8.21   | 0.0053 |
| Y × C × F           | 3                  | 1184.757      | 294.92      | 0.77   | 0.5157 |
| Residuals           | 80                 | 41173.47      | 514.67      |        |        |
| Total               | 95                 | 79913.04      |             |        |        |
| **MBC/MBN**         |                    |               |             |        |        |
| Year                | 3                  | 35.2237       | 11.741      | 17.48  | < 0.0001 |
| Clip                | 1                  | 0.0084        | 0.0084      | 0.01   | 0.911  |
| Year × clip         | 3                  | 0.6657        | 0.2219      | 12.79  | 0.8034 |
| Fertilizer          | 1                  | 8.5921        | 8.5921      | 12.79  | 0.0006 |
| Year × fertilizer   | 3                  | 0.8381        | 0.2793      | 0.42   | 0.742  |
| Clip × fertilizer   | 1                  | 3.7446        | 3.7446      | 5.58   | 0.0206 |
| Y × C × F           | 3                  | 2.96          | 0.9867      | 1.47   | 0.2292 |
| Residuals           | 80                 | 53.7321       | 0.6717      |        |        |
| Total               | 95                 | 105.765       |             |        |        |
| **MR**              |                    |               |             |        |        |
| Year                | 3                  | 128754        | 42918       | 94.63  | < 0.0001 |
| Clip                | 1                  | 18044.83      | 18045       | 39.79  | < 0.0001 |
| Year × clip         | 3                  | 22498.89      | 7499.6      | 16.54  | < 0.0001 |
| Fertilizer          | 1                  | 1404.158      | 1404.2      | 3.1    | 0.0823 |
| Year × fertilizer   | 3                  | 2078.86       | 692.95      | 1.53   | 0.2136 |
| Clip × fertilizer   | 1                  | 15.6736       | 15.674      | 0.03   | 0.853  |
| Y × C × F           | 3                  | 47.9267       | 15.976      | 0.04   | 0.9911 |
| Residuals           | 80                 | 36282.03      | 453.53      |        |        |
| Total               | 95                 | 209126        |             |        |        |
| **$\varphi$CO$_2$** |                    |               |             |        |        |
| Year                | 3                  | 124.758       | 41.586      | 28.3   | < 0.0001 |
| Clip                | 1                  | 13.8548       | 13.855      | 9.43   | 0.0029 |
| Year × clip         | 3                  | 34.0699       | 11.357      | 7.73   | 0.0001 |
| Fertilizer          | 1                  | 0.4388        | 0.4388      | 0.3    | 0.5863 |
| Year × fertilizer   | 3                  | 5.5487        | 1.8496      | 1.26   | 0.2942 |
| Clip × fertilizer   | 1                  | 0.0075        | 0.0075      | 0.01   | 0.9431 |
| Y × C × F           | 3                  | 2.0444        | 0.6813      | 0.46   | 0.7084 |
| Residuals           | 80                 | 117.558       | 1.4695      |        |        |
| Total               | 95                 | 2982801       |             |        |        |
and C cycles tight even under assumed increases in N deposition and fertilization, because mowing in combination with N fertilization increased ANPP but did not affect significantly MBN or other microbial N cycle characteristics. However, in view of the significant effect of the inter-annual variability of the amount of rainfall in our semi-arid study region, management plans must be multi-year, because the key microbial characteristics that were measured in our study fluctuated significantly across years.

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References

Aber, J.D., Nadelhoffe, K.J. & Steudler, A.P. 1989. Nitrogen saturation in northern forest ecosystems. *Biocience*, 32, 378–386.

Bardgett, R.D., Wardle, D.A. & Yeates, G.W. 1998. Linking above-ground and below-ground interactions: growth plant responses to foliar herbivory influence soil organism. *Soil Biology & Biochemistry*, 30, 1867–1878.

Bardgett, R.D., Denton, C.s., Cook, R. 1999. Below-ground herbivory promotes soil nutrient transfer and root growth in grassland. *Ecology Letters*, 2, 357–360.

Berg, B. & Matzner, E. 1997. Effect of N deposition on decomposition of plant litter and soil organic matter in forest systems. *Environments*, 5, 1–25.

Bradley, K., Drijber, R.A. & Knops, J. 2006. Increased N availability in grassland soils modifies their microbial communities and decreases the abundance of arbuscular mycorrhizal fungi. *Soil Biology & Biochemistry*, 38, 1583–1595.

Cabrera, M. & Beare, M.H. 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. *Soil Science Society of American Journal*, 57, 1007–1012.

Christensen, L., Coughenour, M.B., Ellis, J.E. & Chen, Z.Z. 2004. Vulnerability of the Asian typical steppe to grazing and climate change. Climatic Change, 63, 351–368.

Chu, H. & Lin, X.G. 2007. Soil microbial biomass, dehydrogenase activity, bacterial community structure in response to long-term fertilizer management. *Soil Biology & Biochemistry*, 39, 2971–2976.

De Vries, F.T., Bloem, J., Van Eckener, N., Brusaard, L. & Hoffland, E. 2007. Fungal biomass in pastures increases with age and reduced N input. *Soil Biology & Biochemistry*, 39, 1620–1630.

Dijkstra, F.A., Hobbie, S.E., Reich, P.B. & Knops, J.H.M. 2005. Divergent effects of elevated CO2, N fertilization, and plant diversity on soil C and N dynamics in a grassland field experiment. *Plant & Soil*, 272, 41–52.

Elser, J.J., Bracken, M.E.S. & Cleland, E.E. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in fresh water, marine and terrestrial ecosystems. *Ecology Letters*, 10, 1135–1142.

FAO, IUSS Working Group WRB (2007). World Reference Base for Soil Resources, ISRIC, Rome.

Fisk, M.C. & Fahey, T.J. 2001. Microbial biomass and nitrogen cycling responses to fertilization and litter removal in young northern hardwood forests. *Biogeochemistry*, 53, 201–223.

Galloway, J.N., Dentener, F.J. & Capone, D.G. 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry*, 70, 153–226.

Galloway, J.N., Townsend, A.R. & Erisman, J.W. 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science*, 320, 889–892.

Garcia, F.O. & Rice, C.W. 1994. Microbial biomass dynamics in tallgrass prairie. *Soil Science Society of American Journal*, 58, 816–823.

Güsewell, S., Jewell, P.J. & Edwards, P.J. 2005. Effects of heterogeneous habitat use by cattle on nutrient availability and litter decomposition in soils of an Alpine pasture. *Plant & Soil*, 268, 135–149.

Hassink, J. 1992. Effect of grassland management on N mineralization potential, microbial biomass and N yield in the following year. *Netherlands Journal of Agricultural Science*, 40, 173–185.

Hu, S., Chapin, F.S. & Firestone, M.K. 2001. Nitrogen limitation of microbial decomposition in a grassland under elevated CO2. *Nature*, 409, 188–191.

Isteltd, U., Giesler, R. & Nordgren, A. 2003. Changes in soil chemical and microbial properties after a wildfire in a tropical rainforest in Sabah, Malaysia. *Soil Biology & Biochemistry*, 35, 1071–1078.

Johnson, L.C., Shaver, G.R., Cades, D.H., Rastetter, E., Nadelhoffe, K., Giblin, A. *et al.* 2000. Plant carbon-nutrient interactions control CO2 exchange in Alaskan wet sedge tundra ecosystems. *Ecology*, 81, 453–469.

Knapp, A.K., Fay, P.A., Blair, J.M., Collins, S.L., Smith, M.D., Carlisle, J.D. *et al.* 2002. Rainfall variability, carbon cycling, and plant species diversity in a Mesic grassland. *Science*, 298, 2202–2205.

Lebauer, D.S. & Treseder, K.K. 2008. Nitrogen limitations of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology*, 89, 371–379.

Leriche, H., Le, R.X., Gignoux, J., Tuzet, A., Fritz, H., Abbadie, L. *et al.* 2001. Which functional processes control the short-term effect grazing on net primary production in grassland? *Oecologia*, 129, 114–124.

Liu, W., Zhang, Z. & Wan, S. 2009. Predominant role of water in regulating soil and microbial respiration and their responses to climate change in a semi-arid grassland. *Global Change Biology*, 15, 184–195.

Maly, S. & Sarapatka, B. 2002. Seasonal variability in soil N mineralization and nitrification as influenced by N fertilization. *Rostlina Vyroba*, 48, 389–396.

Nohrstedt, H.O., Arnebrant, K., Baath, E. & Soderstrom, B. 1989. Changes in carbon content, respiration rate, ATP content, and microbial biomass in nitrogen fertilized pine forest soils in Sweden. *Canadian Journal of Forest Research*, 19, 323–328.

Pietikäinen, A., Kytövita, M.M. & Vuoti, U. 2005. Mycorrhizal symbiosis and seedling establishment in a subarctic meadow: effects of fertilization and clipping. *Journal of Vegetation Science*, 16, 175–182.

Rousk, J., Brookes, P.C. & Baath, E. 2011. Fungal and bacterial growth responses to N fertilization and pH in the 150-year Park Grass UK grassland experiment. *FEMS Microbiology Ecology*, 76, 89–99.

Shaver, G.R., Canadell, J., Chapin, F.S., Gurevitch, J., Harte, J., Henry, G. *et al.* 2000. Global warming and terrestrial ecosystems: a conceptual framework for analysis. *Bioscience*, 50, 871–882.

Soderstrom, B., Baath, E. & Lundgren, B. 1983. Decrease in soil microbial activity and biomasses owing to nitrogen amendments. *Canadian Journal of Microbiology*, 29, 1500–1506.
Stark, S. & Kytöviita, M.M. 2006. Simulated grazer effects on microbial respiration in a subarctic meadow: implications for nutrient competition between plants and soil microorganisms. *Applied Soil Ecology, 3*, 20–31.

Tix, D., Hebberger, J.A. & Harva, I.C. 2006. Influence of aboveground biomass removal on nitrogen mineralization in a restored tallgrass prairie. *Restoration Ecology, 4*, 561–568.

Treseder, K.K. 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystems studies. *Ecology Letters, 11*, 1111–1120.

Vance, E.D., Brookes, P.C. & Jenkinson, D.S. 1987. An extraction method for measuring soil microbial biomass C. *Soil Biology & Biochemistry, 19*, 703–707.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Linkens, G.E., Matson, P.A., Schindler, D.W. *et al.* 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications, 7*, 737–750.

Wan, S. & Luo, Y. 2003. Substrate regulation of soil respiration in a tallgrass prairie: results of a clipping and shading experiment. *Global Biogeochemical Cycles, 17*, 1–12.

Wang, C.H., Butterbach-Bahl, K., Han, Y., Wang, Q., Zhang, L., Han, X. *et al.* 2011. The effects of biomass removal and N additions on microbial N transformations and biomass at different vegetation types in an old-field ecosystem in northern China. *Plant & Soil, 340*, 397–411.

Xia, J. & Wan, S. 2008. Global response patterns of terrestrial plant species to nitrogen addition. *New Phytologist, 179*, 428–439.

Zak, D.R., Tilman, D., Parmenter, R.R., Rice, C.W., Fisher, F.M., Vose, J. *et al.* 1994. Plant production and soil microorganisms in late-successional ecosystems: a continental-scale study. *Ecology, 75*, 2333–2347.

Zavaleta, E.S., Shaw, M.R. & Chiariello, N.R. 2003. Grassland responses to three years of elevated temperature, CO$_2$, precipitation, and N deposition. *Ecological Monographs, 73*, 585–604.