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 $10 \text{ g N m}^{-2} \text{ year}^{-1}$ as NH_4NO_3 to mown and unmown plots ($4 \times 4 \text{ m}^2$) and *in situ* rates of net ammonification (R_{amm}), nitrification (R_{nit}) 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_{nit} , 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 $q\text{CO}_2$ 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_{amm} 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 $2\text{--}5 \text{ g m}^{-2} \text{ year}^{-1}$ 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,

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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

Figure 1 Seasonal dynamics of soil temperature ($^{\circ}\text{C}$, ST) and moisture (% SM) in 2006, 2007, 2008 and 2009. CC, control; CN, N addition; FC, mowing; FN, mowing plus N addition.

respiration. If a significant inter-annual variability was demonstrated (year effect $P < 0.05$), repeated measures ANOVA (RMANOVA) was used to examine the mowing and N addition effects on microbial N transformation for each growing season for each year (Tables 2, 3). After observing that the interaction between the data and treatment was significant, the effect of the treatment was tested separately. The responses of soil properties (microbial N turnover, microbial biomass and inorganic N content) to mowing or N addition or a combination of both were tested with a two-way ANOVA. All the data were statistically analysed with SAS V.8.1 (SAS Institute Inc., Cary, NC, USA).

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

Table 1 Soil physiochemical properties (0–10 cm) and aboveground (ANPP) and belowground (BNPP) biomass (N = 6, mean $\pm$ 1 SE)

Source of variation	Control	N addition	Mowing	N addition + mowing
Total organic carbon / g kg ⁻¹	10.00 $\pm$ 0.50	11.4 $\pm$ 0.30	13.9 $\pm$ 0.5	15.00 $\pm$ 0.80
Total nitrogen / g kg ⁻¹	1.40 $\pm$ 0.05	1.40 $\pm$ 0.03	1.30 $\pm$ 0.05	1.50 $\pm$ 0.06
C:N ratio	7.33 $\pm$ 0.51	8.19 $\pm$ 0.24	10.46 $\pm$ 0.42	10.22 $\pm$ 0.41
pH value	7.50 $\pm$ 0.12	6.92 $\pm$ 0.11	7.60 $\pm$ 0.05	6.86 $\pm$ 0.09
ANPP / g m ⁻²	219.17 $\pm$ 23.12	313.83 $\pm$ 24.61	194.48 $\pm$ 15.30	296.33 $\pm$ 20.60
BNPP / g m ⁻²	930.17 $\pm$ 108.22	1348.10 $\pm$ 113.2	898.64 $\pm$ 66.43	977.92 $\pm$ 70.39

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

Figure 2 Peak above-ground biomass (ANPP) and standing litter in 2006, 2007, 2008 and 2009 (mean $\pm$ SE, $n = 6$). CC, control; CN, N addition; FC, mowing; FN, mowing plus N addition the upper panel-ANPP, the middle panel-Litter, the bottom panel-ANPP+Litter.

($P > 0.05$). 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

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 $\times$ mowing and year $\times$ 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 $\times$ mowing $\times$ 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 $q\text{CO}_2$ 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 $q\text{CO}_2$ 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 $q\text{CO}_2$.

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 & Sarapatka, 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 arbuscular mycorrhizal fungi

Table 2 Summary table for repeated ANOVA of soil temperature (ST) and soil moisture (SM), ammonification rate (R_{amm}), nitrification rate (R_{nit}), and net N mineralization rate (R_{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 $\times$ clip	3	5.777	1.926	1.98	0.1284
Fertilizer	1	0.863	0.863	0.87	0.3529
Year $\times$ fertilizer	3	0.1831	0.0061	0.06	0.9798
Clip $\times$ fertilizer	1	0.38	0.38	0.38	0.5369
Y $\times$ C $\times$ 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 $\times$ clip	3	3.657	1.219	0.51	0.6767
Fertilizer	1	9.601	9.601	4.01	0.0485
Year $\times$ fertilizer	3	14.2749	4.7583	1.99	0.1221
Clip $\times$ fertilizer	1	35.4051	35.4051	14.81	0.0002
Y $\times$ C $\times$ 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 $\times$ clip	3	0.1498	0.0499	1.5	0.2206
Fertilizer	1	0.03654	0.03654	1.1	0.2978
Year $\times$ fertilizer	3	0.2334	0.0778	2.34	0.0798
Clip $\times$ fertilizer	1	0.1456	0.1456	4.38	0.0396
Y $\times$ C $\times$ 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 $\times$ clip	3	0.2874	0.0958	8.01	< 0.0001
Fertilizer	1	0.2517	0.2517	21.05	< 0.0001
Year $\times$ fertilizer	3	0.4134	0.1378	11.52	< 0.0001
Clip $\times$ fertilizer	1	0.0226	0.0226	1.89	0.1728
Y $\times$ C $\times$ 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 $\times$ clip	3	0.8403	0.2801	3.9	0.0118
Fertilizer	1	0.673	0.673	9.36	0.003
Year $\times$ fertilizer	3	0.9019	0.3006	4.18	0.0084
Clip $\times$ fertilizer	1	0.2797	0.2797	3.89	0.052
Y $\times$ C $\times$ F	3	0.8952	0.2984	4.15	0.0087
Residuals	80	5.7507	0.0718		
Total	95	15.5897

Figure 3 Seasonal dynamics of ammonification (Ramm), nitrification (Rnit) and net N mineralization (Rmin) rates (mean $\pm$ 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 $q\text{CO}_2$ 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

Figure 4 Effects of N addition and clipping on soil microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and the ratio of MBC:MBN in 2006, 2007, 2008 and 2009, respectively (mean $\pm$ SE, $n = 6$). CC, control; CN, N addition; FC, mowing; FN, mowing plus N addition.

the form of root exudates and the production of new below-ground plant root litter (Bargett *et al.*, 1999).

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

Figure 5 Effects of N addition and clipping on soil microbial respiration (MR) and $q\text{CO}_2$ in 2006, 2007, 2008 and 2009 (mean $\pm$ SE, $n = 6$). CC, control; CN, N addition; FC, mowing; FN, mowing plus N addition.

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 & Kytöviita (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 $q\text{CO}_2$

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	P
MBC					
Year	3	6 888 080	229 360	55.93	< 0.0001
Clip	1	15988.52	15 989	3.9	0.0518
Year $\times$ clip	3	2878.062	959.35	0.23	0.8725
Fertilizer	1	22019.32	22 019	5.37	0.0231
Year $\times$ fertilizer	3	1947.46	615.82	0.15	0.9293
Clip $\times$ fertilizer	1	464.2441	464.24	0.11	0.7374
Y $\times$ C $\times$ F	3	15254.06	5084.7	1.24	0.3008
Residuals	80	328072.3	4100.9		
Total	95	1 074 605			
MBN					
Year	3	26439.47	8813.2	17.12	< 0.0001
Clip	1	2042.784	2042.8	3.97	0.0498
Year $\times$ clip	3	930.001	310	0.6	0.6154
Fertilizer	1	2607.08	2607.1	5.07	0.0272
Year $\times$ fertilizer	3	1309.246	436.42	0.85	0.4717
Clip $\times$ fertilizer	1	4226.229	4226.2	8.21	0.0053
Y $\times$ C $\times$ 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 $\times$ clip	3	0.6657	0.2219	12.79	0.8034
Fertilizer	1	8.5921	8.5921	12.79	0.0006
Year $\times$ fertilizer	3	0.8381	0.2793	0.42	0.742
Clip $\times$ fertilizer	1	3.7446	3.7446	5.58	0.0206
Y $\times$ C $\times$ F	3	2.96	0.9867	1.47	0.2292
Residuals	80	53.7321	0.6717		
Total	95	105.765			
MR					
Year	3	128 754	42 918	94.63	< 0.0001
Clip	1	18044.83	18 045	39.79	< 0.0001
Year $\times$ clip	3	22498.89	7499.6	16.54	< 0.0001
Fertilizer	1	1404.158	1404.2	3.1	0.0823
Year $\times$ fertilizer	3	2078.86	692.95	1.53	0.2136
Clip $\times$ fertilizer	1	15.6736	15.674	0.03	0.853
Y $\times$ C $\times$ F	3	47.9267	15.976	0.04	0.9911
Residuals	80	36282.03	453.53		
Total	95	209 126			
$q\text{CO}_2$					
Year	3	124.758	41.586	28.3	< 0.0001
Clip	1	13.8548	13.855	9.43	0.0029
Year $\times$ clip	3	34.0699	11.357	7.73	0.0001
Fertilizer	1	0.4388	0.4388	0.3	0.5863
Year $\times$ fertilizer	3	5.5487	1.8496	1.26	0.2942
Clip $\times$ fertilizer	1	0.0075	0.0075	0.01	0.9431
Y $\times$ C $\times$ F	3	2.0444	0.6813	0.46	0.7084
Residuals	80	117.558	1.4695		
Total	95	298.2801			

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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