Plant Soil (2011) 340:397–411
DOI 10.1007/s11104-010-0611-z

REGULAR ARTICLE

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

Changhui Wang · Klaus Butterbach-Bahl · Yi Han · Qibing Wang · Lihua Zhang · Xingguo Han · Xuerong Xing

Received: 22 May 2010 / Accepted: 11 October 2010 / Published online: 20 October 2010

Abstract There is an increasing demand for the sustainable management of old-field communities in northern China, which have developed on abandoned cropland on formerly converted natural steppe sites, to regain forage yield, biodiversity, and soil fertility. In thus study we examined how two management options—clipping and nitrogen (N) addition—may affect net >microbial N mineralization (ammonification+nitrification), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and microbial respirations (MR) in grass dominated, herb dominated, and grass-herb mixed patches in an old-field community in northern China. Topsoil (0–10 cm) net N mineralization rate was 177% and 69% higher in mixed grass and herb patches (patch B) as compared to unmixed grass (patch A) or herb (patch C) patches, respectively. Topsoil MBN was significantly different among the three patches with the highest value for soils taken from umixed grass patches. However, patches with mixed grass and herb or herb dominated patches had 12% higher microbial respiration (MR) than unmixed grass patch. Clipping and N addition had no effects on net N mineralization or MBC, but both treatments decreased MBN and MR and increased the ratio between microbial biomass C and microbial biomass N (MBC/MBN) in the growing season. Incubation of soil cores under optimal water and temperature conditions in the laboratory showed that the response of microbial N transformations in soils under different vegetation patches to experimental N addition and clipping was limited by soil water availability. Our results strongly highlight the need to further study the importance of belowground C supply as a control of microbial N cycling processes. It also suggests that during the restoration process of degenerated croplands N cycling rates are stimulated, but that the magnitude of this stimulation is modulated by plant community composition of the old-fields.

Keywords Clipping · Microbial biomass · N addition · N mineralization · Nitrification · Old field · Patch

Responsible Editor: Ute Skiba.

Electronic supplementary material The online version of this article (doi:10.1007/s11104-010-0611-z) contains supplementary material, which is available to authorized users.

C. Wang · Y. Han · Q. Wang · L. Zhang · X. Han · X. Xing (✉)
Key Laboratory of Vegetation and Environmental Change, Institute of Botany, The Chinese Academy of Sciences, Beijing 100093, China
e-mail: xrxing@cashq.ac.cn

C. Wang
e-mail: wangch@ibcas.ac.cn

K. Butterbach-Bahl
Institute for Meteorology and Climate Research, Atmospheric Environmental Research, Garmisch-Partenkirchen 82467, Germany

Introduction

Nitrogen (N) is an important growth-limiting nutrient in terrestrial ecosystems (Vitousek and Howarth

1991). N cycling is controlled by interactions among physical and biological processes that operate across a wide range of spatial and temporal scales (Vitousek et al. 1997; Hook and Burke 2000). However, human activity has now more than doubled N fixation rates relative to natural rates. There is much evidence that the enrichment of terrestrial ecosystems with reactive N not only affects plant productivity (Lee et al. 2010) and plant species composition (Stevens et al. 2004; Bobbink et al. 2010) but also soil microbial activity (Schmidt et al. 2004).

Plant species communites influence soil nutrient availability through their effects on litter decomposition and nutrient uptake, input, and loss (Hobbie 1992; Eviner and Chapin 2004; Dijkstra et al. 2005). Microbial biomass and microbial N transformations are key components of plant-soil feedbacks and, thus, are known to respond to variations in nutrient availability (Fisk and Fahey 2001; Manning et al. 2008a). Soil microbial N transformations processes such as mineralization and nitrification are key drivers for the cycling of N and other nutrients in ecosystems (Lovell et al. 1995). Microbial biomass has been found to be a good general indicator of N cycling processes at landscape and regional scales (Cookson et al. 2005). However, to better understand how nutrient availability may affect the competition of plants and microorganisms for soil nutrients and the magnitude of microbial N turnover processes experimental studies are needed.

In natural grasslands, management activities can influence soil fertility directly through fertilizer inputs (such as N addition, Makarov et al. 2003) and via management-induced changes (such as litter removal) in plant composition and litter C input (Patra et al. 2005). N availability to plants and microbes varies with environmental conditions and changes in land use (Vitousek and Howarth 1991). Total and mass-specific litter decomposition may decrease with clipping but increase with N addition because of increased plant production and, thus, litter inputs (Melillo et al. 1982; Manning et al. 2006; 2008a, b). Additions of inorganic N can inhibit the decomposition of soil organic matter, for the lack of microbial mining of recalcitrant C for N when N is added (Güsewell et al. 2005; Berg and Matzner 1997). Clipping accelerates the N cycle (Chu and Lin 2007) and promotes increased aboveground and belowground plant growth (Leriche et al. 2001) and root exudation (Zhang and Zak 1998; Hamilton and Frank 2001). However, the influence of clipping, N addition and their interaction on soil microbial activity and N dynamics has not been explored in semi-arid grasslands of northern China.

Northern China is largely covered by steppe ecosystems, which are representing the typical arid and semiarid regional vegetation type that covers a vast area of the Eurasian continent. During the past half century, rapid increases in human populations and cropping pressure have severely degraded the temperate steppe and have caused declines in primary productivity, species richness, and soil organic carbon content. Significant parts of the Northern China steppe have been converted in the recent past to arable land. However, due to degradation, yields have declined and cropland has been abandoned forming so-called old-field communities. Old-field habitats are more sensitive to climatic change and management than natural grasslands because it is an unstable successional system in succession (Ihori and Burke 1995, Uri et al. 2008). In addition political and economic changes also caused a significant increase in old-field communities in Northern China (Kang et al. 2007). In order to maintain the sustainability of the temperate steppe in terms of forage yield, biodiversity, and soil fertility, the Chinese government has recently expended great effort to restore the degraded temperate steppe and to abandon its use as cropland. N availability and transformation processes are key parameters for the evaluation of this management practice because they greatly affect steppe productivity and species richness (Barger et al. 2004). However, little information is available on in situ soil N availability and mineralization and microbial activity in the old-field communities of Northern China. Plant community composition, N addition and clipping can all influence spatial patterns of N transformation and microbial activity (Robson and Lavorel 2007). Although each of these factors has been shown to be important in specific cases, no study evaluated their relative importance and interaction in old-fields of China. We therefore designed an experiment in an old-field ecosystem of northern China to determine how microbial biomass, in situ net N mineralization and microbial activity is affected by changes in nutrient availability (N addition) and clipping (aboveground biomass removal) for three different plant communities: a grass dominated, grass-herb mixture and herb dominated. We also compared mineralization patterns between in situ and laboratory incubations to evaluate

the role of environmental conditions on N supply across management treatments and microsites.

Materials and methods

Site description

The study was carried out in an experimental area located in northern China, Duolun county, 42°27′N and 116°40′E. The mean annual precipitation from 1953 to 2006 as measured at the meteorological station at Duolun Restoration Ecology Grassland Station was 382.2 mm with more than 80% of rainfall occurring from mid-June to late September. The mean annual temperature is 2.1°C (Niu et al. 2009). In Duolun county large parts of typical temperate steppe has been converted to cropland in the 1960s. In a restoration attempt some of the cropland was abandoned in 1995 without any further management. Three principal types of vegetation patches, dominated by different plant species, subsequently developed on these abandoned croplands: Patch type A (in the following Patch A) is dominated by grasses and here specifically *Pennisetum centrasiaticum* (grass) (>85%); Patch B is a mixture of grasses and herb with *Pennisetum centrasiaticum* and *Artemisia frigida* dominating (together >50%) Patch C is dominated by herb with *Artemisia frigida* (>50%) being the most prominent one herb. The soil at our sites are classified as chestnut soils (Chinese classification) or Calcic Luvisols according to the FAO classification with 62.75±0.04% sand, 20.30±0.01% silt, and 16.95±0.01% clay in top 20 cm of the soil, respectively. Soil bulk density and pH values in the top 10 cm of the soil are 1.31±0.02 g cm^{-3} and 6.84±0.07, respectively. Soil organic C and total N contents in the topmost 10 cm of the mineral soil are 16.10±0.89 gkg^{-1}, and 1.48±0.10 gkg^{-1}, respectively. Aboveground biomass as well as belowground biomass was highest in the grass patches and lowest in the herb patches, with belowground biomass being a factor of 3–4 times higher as aboveground biomass.

Experimental design

For each patch type twenty-four 4 m×4 m plots were established in 2005, i.e. 10 years after abandonment of agriculture. The distance between the blocks of the three patches was approximately 4 m (Fig. 1). Four different treatments were applied randomly to each block: a) control, b) addition of nitrogen fertilizer, c) clipping of aboveground biomass and d) a combination of clipping and nitrogen fertilization. Clipping was performed in late August 2006, 2007, and 2008—i.e. at the end of the growing season—in such a way that all biomass >3 cm (green biomass+standing litter) was taken away. In the N treatments, NH_4NO_3 granulate was applied in mid-July 2006, 2007, and 2008 at a rate of 10 gNm^{-2}.

Topsoil (0–10 cm mineral soil) soil samples were taken in monthly intervals from all 72 plots from May–October 2008 (13 May, 19 June, 19 July, 18 August, 17 September, and 19 October) for determination of net nitrogen turnover, microbial biomass C and N as well as for measurements of microbial respiration (see below). Soil temperature at a depth of 10 cm was measured between 9:00–10:00 twice a month from May to October in each plot using a soil thermometer (Longstem Thermometer 6310, Spectrum Technologies, Plainfield, USA). Soil moisture (0–10 cm) was measured in each plot gravimetrically once a month during the sampling period from May to October.

Soil sampling and field incubation

For measurements of in situ net N mineralization during the growing season in 2008 we used the buried soil core technique as described by Raison et al. (1987). At each sampling date, in each plot and following the removal of aboveground living plants and litter, four sharpened PVC cylinders (5 cm in diameter and 12 cm in length) were randomly driven 10 cm into the soil adjacent to each other (<5 cm in distance). Two cylinders were immediately removed, whereas the other two remained for another 30 days in the field. The latter cores were covered with 0.01-mm thick polyethylene film to minimize evaporation during the 30 days in-situ incubation. Following sampling, soil cores were immediately processed at a laboratory located at Duolun research station. I.e. the soil was mixed, stones and coarse roots removed and finally soil was sieved with 2 mm mesh and stored at 4°C until further analyses (<48 h) in the laboratory of the Institute of Botany in Beijing.

To analyze soil inorganic N concentrations, a 10-g aliquot subsample was taken from each sieved soil

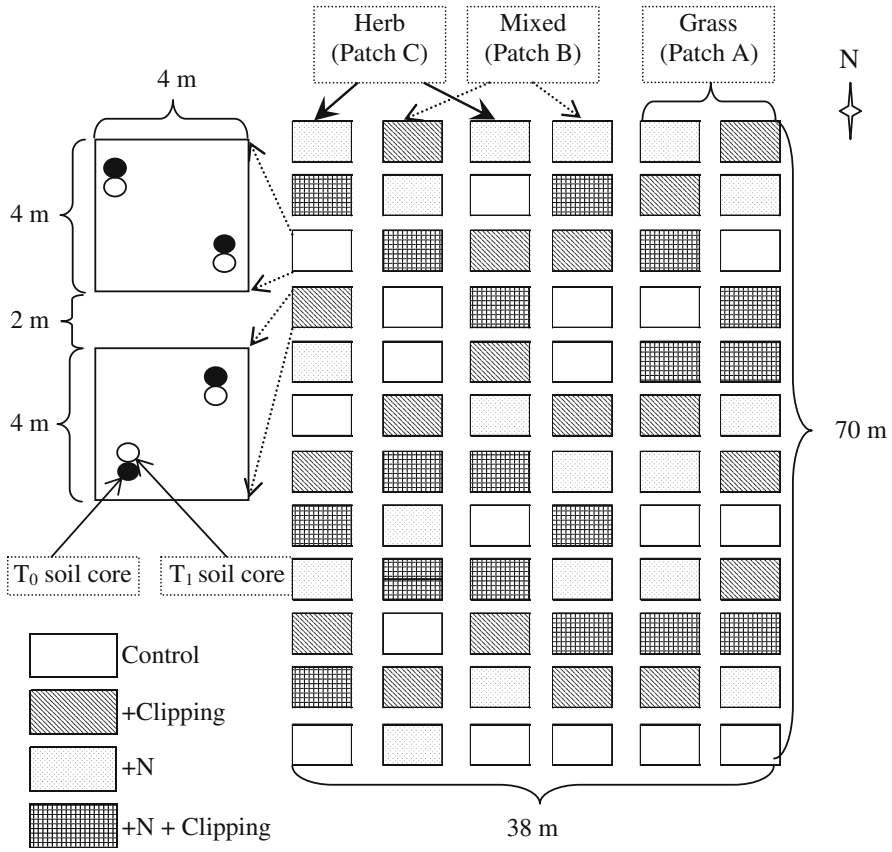

Fig. 1 Experimental design

sample and extracted with 50 ml of 2 mol L^{-1} KCl solution. The soil suspension was rigorously shaken for 1 h in a reciprocal shaker and thereafter filtered through Whatman No. 1 filter paper (12.5 cm in diameter). Soil solutions were immediately analyzed for NH_4^+-N and NO_3^--N on a FIAstar 5000 Analyzer (Foss Tecator, Denmark). Net microbial N turnover rates were calculated from differences in soil NH_4 and NO_3 concentrations between days 0 and 30 with mineralization rates being expressed on a dry mass basis.

Potential net N mineralization and microbial analysis

For all soil samples from each plot we measured potential net N mineralization in the top 10 cm of the soil by incubating subsamples of sieved soil in a plastic jar covered with 0.01 mm thick polyethylene film to minimize evaporation 1 week in the laboratory under constant and optimum temperature (25°C) and soil moisture conditions. For this soil subsamples from the two cores taken in each plot were pooled and subsequently sub-sampled for further analysis (N=3): inorganic N concentration, incubations for potential net N mineralization, determination of microbial biomass C and N and soil microbial respiration. NO_3^- and NH_4^+ were determined from 10-g subsamples; these samples were extracted in 50 ml of 2 molL^{-1} KCl (see above). Potential net N mineralization was calculated as the difference between the final and initial inorganic N content of the soil.

Microbial biomass was measured using the fumigation-extraction method (Vance et al. 1987). Briefly, the fresh soil samples were carefully adjusted to 60–70% of field water-holding capacity in the dark and incubated for 1 week at 25°C. Then the moist samples (15-g dry weight equivalent) were fumigated for 24 h with ethanol-free $CHCl_3$ (Vance et al. 1987). Soil extracts from the fumigated and controlled samples were obtained by shaking soil samples with 50 ml of 0.5 M K_2SO_4 for 30 min. Extracts were filtered through 0.45-μm filters and frozen at −20°C before analysis of extractable C and N by dichromate digestion and Kjeldahl digestion, as described by Lovell et al. (1995). Microbial biomass C and N were calculated from the difference between extractable C

and N contents in the fumigated and controlled samples using conversion factors (k_{EC} and k_{EN}) equal to 0.38 and 0.45 (Lovell et al. 1995), respectively. All results were expressed on an oven-dried soil basis (105°C, 24 h).

Microbial respiration was measured by alkali absorption of CO_2 evolved at 25°C and optimal soil moisture for 7 days followed by titrating the residual OH^- with a standardized acid (Hu and Bruggen 1997). Briefly, 25 g fresh soil was incubated in a 500-ml glass flask. The glass flask was connected with a glass tube (6 cm in diameter) in which 5 ml of 0.05 M NaOH solution was injected to capture the CO_2 produced by the soil microbes. The metabolic quotient (qCO_2) was calculated from the estimates of microbial respiration and microbial biomass carbon as follows: [(milligram CO_2-C evolved in 7 days kg^{-1} soil) / (milligram microbial biomass C kg^{-1} soil) / (7 days × 24 h) × 1,000], and thus the qCO_2 was expressed as mgCO_2-C g^{-1} $C_{microbial}$ h^{-1} (Wardle and Ghani 1995). All microbial indices were calculated on a mass basis of oven-dried soil.

Soil organic C was measured using the potassium dichromate-vitriol oxidation method (Lavian et al. 2001). Soil total N was measured by Kjeldahl digestion (Cabrera and Beare 1993).

The ratio between in-situ measured field N mineralization rates and those measured under controlled conditions in the laboratory

To index possible environmental constraints on N mineralization, we calculated the ratio between in-situ measured field N mineralization rates and those measured under controlled conditions in the laboratory. This ratio can be considered an index of environmental limitations to N mineralization in the field (the lower the ratio, the greater the environmental limitation) since laboratory incubations are conducted at relatively optimal temperature (25°C) and moisture levels (70% of maximum water holding capacity).

Statistical analysis

Differences in soil characteristics and plant biomass among the three patches were examined using a one-way analysis of variance (ANOVA). A repeated measures ANOVA (RM-ANOVA) was used to determine the main and interactive effects of patch, clipping, and N addition on soil N transformation and microbial measures (Tables 1 and 2). After observing that the interaction between data and treatment (N addition and clipping) was significant, the effect of treatment was tested separately according to the sampling date. The averages of the 6 months of data were used to analyze the effects of treatments generally using a nested-factorial design and regression. All ANOVAs were followed by $LSD_{0.05}$ (least significant difference) post-hoc test to determine the minimum difference between a pair of means necessary for statistical significance. A General Linear Model (GLM) with a Duncan test was used to examine the main effects of patch, clipping, N addition, and their possible interactions on the seasonal mean values. To examine the responses of soil and microbial parameters (microbial N turnover, microbial biomass, inorganic N content) in different patches to clipping or N addition or a combination of both, a two-way ANOVA was applied. Simple and multiple linear and nonlinear regression analyses were used to examine the relationships between N mineralization and soil total N content, microbial biomass N, extractable N, and aboveground biomass. All statistical analyses were performed using SAS V.8.1 (SAS Institute Inc. Cary, NC, USA).

Results

Soil temperature and moisture content during the growing season in 2008

In average, the herb patches had the highest soil temperature over the entire vegetation periods ($P<0.001$). Clipping significantly increased average soil temperature by 0.4°C ($P<0.001$) and decreased soil moisture by 10% ($P<0.0001$). Soil moisture was lowest for mixture and herb patches in May and for grass patches in June (Fig. 2). During these months soil moisture values in the uppermost 10 cm of the topsoil were <5%. Mean soil moisture in grass dominated patches was significantly ($P<0.0001$) by 18% and 35% higher than in mixed or herb dominated patches, respectively.

Soil physiochemical properties and plant biomass in the three patches

There were no differences in soil organic C and the C/N ratio among the three vegetation patches, but soil

Table 1 Results (*F* values) of repeated-measures ANOVA for inorganic N pool and microbial N transformations

Source of variation	df	Inorganic N	R_{amm}	R_{nit}	R_{min}
Vegetation Patch (P)	2	1.57 ns	13.22***	2.36 ns	7.70***
Clipping (C)	1	2.43 ns	0.33 ns	1.49 ns	0.80 ns
N addition (N)	1	366.93***	1.69 ns	0.17 ns	1.41 ns
P×C	2	1.26 ns	2.81 ns	5.48**	4.56**
P×N	2	6.33**	18.15***	1.03 ns	13.96***
C×N	1	3.60 ns	5.92*	0.42 ns	4.68*
P×C×N	2	1.66 ns	1.21 ns	3.60**	2.44 ns
Sampling Date (D)	4	124.15***	18.15***	23.26***	11.94***
D×P	8	7.88***	7.21***	1.71 ns	5.07***
D×C	4	1.11 ns	0.08 ns	1.55 ns	0.35 ns
D×N	4	87.49***	3.25*	15.96***	6.88***
D×P×C	8	1.26 ns	4.26***	3.84***	5.26 ns
D×P×N	8	7.56***	20.57***	5.45***	17.08***
D×C×N	4	1.60 ns	5.25***	1.03 ns	4.50**
D×P×C×N	8	1.01 ns	1.02 ns	0.77 ns	1.11 ns

*R*amm ammonification rate, *R*nit nitrification rate, *R*min net N mineralization rate

*, **, and ***: statistically significant at $P<0.05$, 0.01, and 0.001, respectively; ns: statistically insignificant at $P<0.05$

total N in the herb patches was significantly lower than that in grass and mixed patches ($P<0.001$). N addition had significantly decreased soil pH values by 9.3%, 5.2%, and 8.8% in all patches ($P<0.05$). N addition also significantly enhanced the peak aboveground biomass by 60% and 40% in grass (437±21 $g m^{-2}$) and mixed patches (385±27 $g m^{-2}$), respectively ($P<0.001$), but had no effect in herb patches (283±30 $g m^{-2}$). Furthermore, N addition had significantly increased belowground biomass by 45% in grass patches ($P<0.001$), whereas such an effect could not be demonstrated for the other vegetation patches ($P>0.05$, Table 1).

Table 2 Results (*F* values) of repeated-measures ANOVA for microbial indices

Source of variation	df	Extra C	Extra N	MBC	MBN	MBC/MBN	MR	qCO_2
Vegetation Patch (P)	2	19.48***	8.37***	1.74 ns	31.45***	58.31***	21.00***	37.46***
Clipping (C)	1	2.16 ns	2.96 ns	3.10 ns	26.75***	65.48***	15.76***	10.89**
N addition (N)	1	9.73**	63.67***	0.03 ns	67.69***	56.66***	11.39***	2.88 ns
P×C	2	2.68 ns	2.21 ns	0.71 ns	2.23 ns	6.67**	0.23 ns	0.73 ns
P×N	2	0.97 ns	3.80*	1.03 ns	1.09 ns	0.92 ns	0.88 ns	13.72***
C×N	1	3.44 ns	0.33 ns	4.63 ns	0.76 ns	2.27 ns	1.15 ns	13.09***
P×C×N	2	8.57***	6.68**	1.43 ns	9.57***	6.82**	1.72 ns	2.41 ns
Sampling Date (D)	5	220.27***	29.10***	65.31***	114.7***	81.64***	45.09***	297.33***
D×P	10	10.23***	12.29***	4.53***	12.7***	12.59***	4.78***	33.67***
D×C	5	3.8**	5.86***	1.99 ns	0.91 ns	8.21***	5.49***	6.88***
D×N	5	0.57 ns	9.34***	1.04 ns	1.74 ns	2.19 ns	1.21 ns	3.39**
D×P×C	10	5.81***	1.59 ns	3.12***	4.33***	4.43***	2.82**	11.09***
D×P×N	10	2.83**	10.30***	2.70**	3.39***	2.42**	3.76***	6.12***
D×C×N	5	0.69 ns	2.27*	1.26 ns	1.70 ns	1.58 ns	0.29 ns	15.08***
D×P×C×N	10	2.53**	1.54	2.21*	3.63***	2.92**	3.63***	2.28*

ExC Soil extractable C, *ExN* soil extractable N, *MBC* microbial biomass carbon, *MBN* microbial biomass nitrogen, *MR* microbial respirations

*, **, and ***: statistically significant at $P<0.05$, 0.01, and 0.001, respectively; ns: statistically insignificant at $P<0.05$

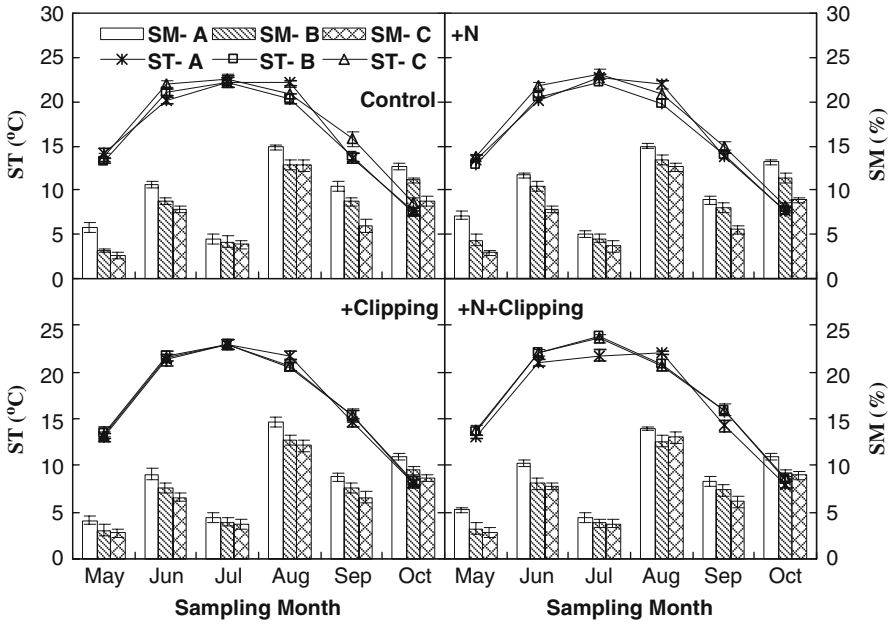

Fig. 2 The effects of clipping and N addition on soil moisture content (%, SM, bars in panels) and soil temperature (°C, ST, lines in panels) in three patches (A-grass, B-mixed (grass+ herb), C-herb) during the growing season (mean ±1 SE) in 2008

Soil inorganic N pool and N transformation in the three patches during the growing season in 2008

For all patches significant seasonal variations ($P<0.001$) in soil inorganic N over the growing season were observed (Table 1, Fig. 3), though differences among the three patches or in response to clipping could not be demonstrated (both $P>0.05$). However, N addition significantly increased the soil inorganic N pool by 113.3% ($P<0.001$) over the growing season.

All microbial soil N transformation indices measured in this study showed strong seasonal fluctuations in 2008 (all $P<0.001$; Table 1, Fig. 4). Ammonification and N mineralization rates (both $P<0.001$), but not nitrification rates ($P>0.05$), were variable among the three vegetation patches (Table 1, Fig. 4). Specifically, the microbial ammonification rate was 461% ($P<$

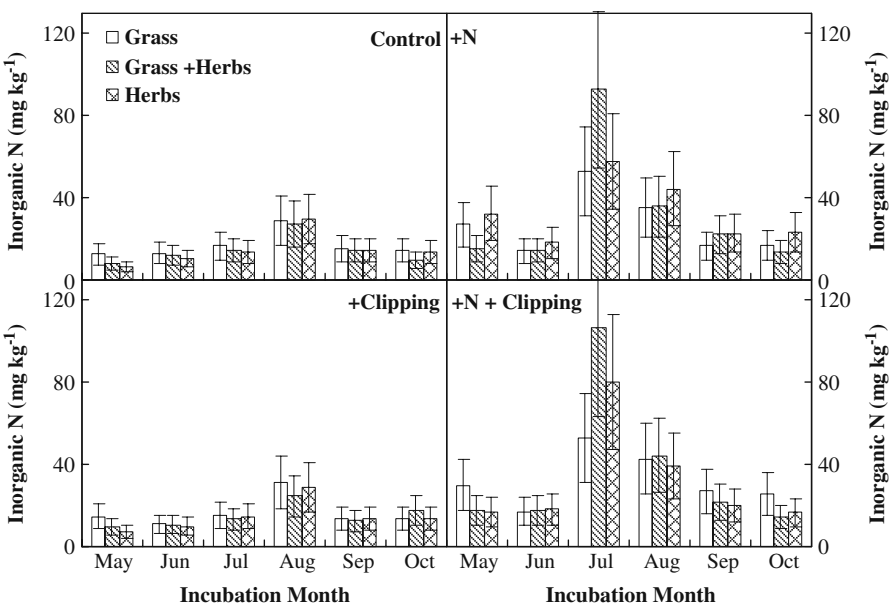

Fig. 3 The effects of clipping and N addition on the inorganic N pool in soils (0–10 cm) of different vegetation patches during the growing season (mean±1 SE) in 2008

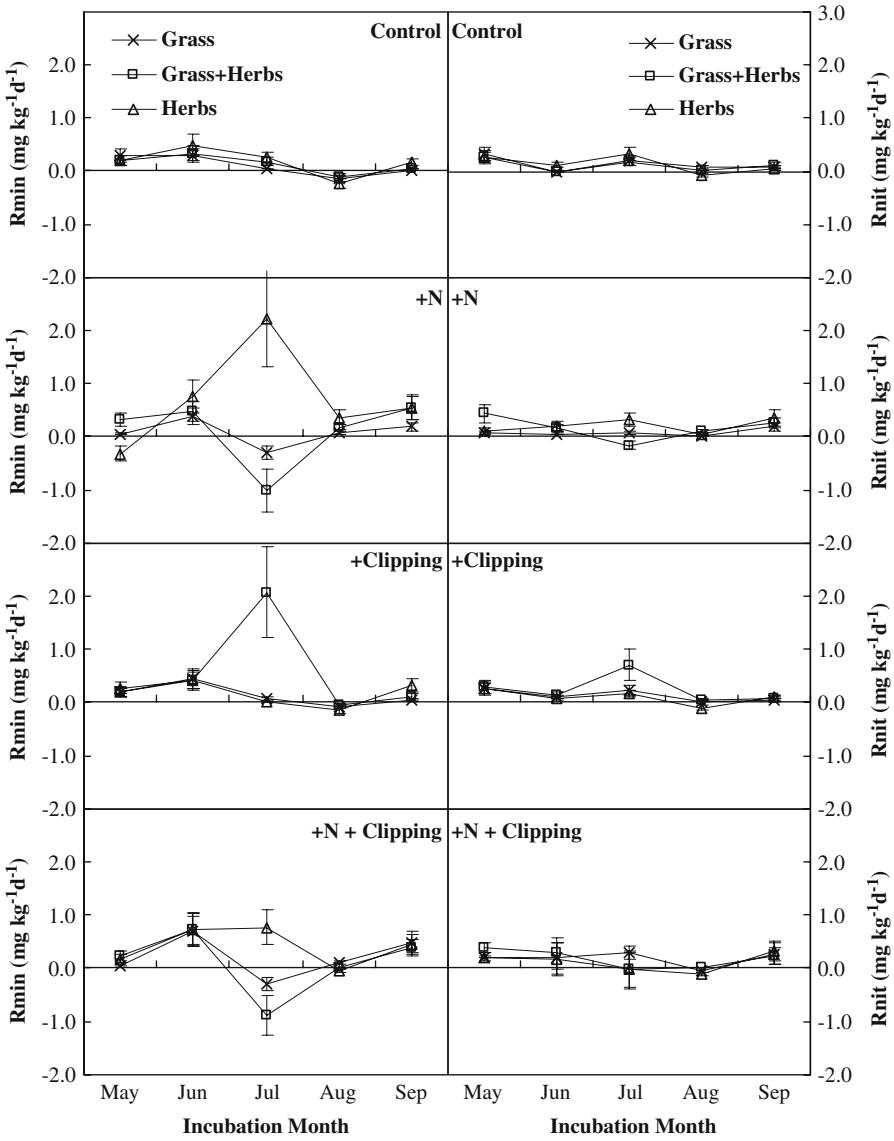

Fig. 4 The effects of clipping and N addition on net N mineralization (Rmin) and net nitrification (Rnit) rates in soils (0–10 cm) of different vegetation patches over the growing season (mean±1 SE)

0.001) greater in the herb patches than in grass/herb mixed patches (Fig. 4). Net N mineralization rate in herb patches was 177% and 69% of the rates in grass and mixed patches, respectively ($P<0.001$).

Clipping and N addition had no effects on N transformation among the three patches ($P>0.05$, Table 1). However, patch significantly interacted with N addition ($P<0.001$). For ammonification and net N mineralization rates significant differences were found between individual sampling dates ($P<0.001$), while there was no effect of sampling date on nitrification rate ($P>0.05$). However, sampling date and its interaction with patch and N addition had significant effects on soil ammonification, nitrification and net N mineralization rates ($P<0.001$).

There were interaction effects of clipping and N addition on ammonification and net N mineralization rates even though the main effects of clipping or N addition were not significant ($P<0.05$). No interactive effect between sampling date and clipping on N transformation was found ($P>0.05$). However, the interaction between sampling date and N addition affected the ammonification rate ($P<0.05$), nitrification rate ($P<0.001$) and net N mineralization rate ($P<0.001$). The three-way interactions among sampling date and clipping and N addition had significant

effects on the soil ammonification rate ($P<0.001$) and the net N mineralization rate ($P<0.01$).

The ratio of the net mineralization rate between in situ incubation and the laboratory incubation was significantly different ($P<0.05$). The ratio in mixed patches was significantly higher than in grass and herb patches ($P<0.05$) and significantly higher in grass patches than in herb patches.

Soil extractable C and N pool in the three patches during the growing season in 2008

Extractable C varied significantly during the growing season in 2008 ($P<0.001$, Table 2, Fig. 5) and between vegetation patches. Soil extractable C was on average, 18% ($P<0.001$) and 21% ($P<0.001$) higher in herb patches than in mixed and grass patches, respectively. Clipping had no statistically effect on soil extractable C ($P>0.05$), whereas N addition significantly increased soil extractable C by 9.0% ($P<0.01$). There were also significant effects of the interactions of sampling date with patch, clipping, N addition and sampling date which are documented in Table 2.

In addition to differences in extractable C we also found significant seasonal differences in soil extractable N across the growing season ($P<0.001$) in 2008. Soil extractable N was on average, 14.8% and 24.6% higher in grass patches than in mixed and herb patches ($P<0.001$), respectively, and 8.5% higher in mixed patches than in herb patches. N addition and clipping enhanced soil extractable N by 33% ($P<0.001$) and 7.3% ($P<0.05$) over the growing season, respectively. For further interactive significant effects see (Table 2).

Microbial measures in the three patches during the growing season in 2008

All soil microbial indices (MBC, MBN, MBC/MBN, MR, qCO_2) measured in this study showed strong seasonal fluctuations (Table 2, Fig. 5, Fig. 6). Over the 2008 growing season, there were significant differences in microbial biomass N (MBN) ($P<0.001$) and the ratio of microbial C:N (MBC/MBN) ($P<0.001$) between the different vegetation patches. Soil MBN levels were 5.2% ($P<0.001$) and 19.7% ($P<0.001$) greater in grass than in mixed and herb patches and 13.8% ($P<0.001$) greater in mixed patches than in herb patches, respectively. The ratios of MBC/MBN were 18% and 27% higher in herb than in grass and mixed patches ($P<0.001$). Clipping and N addition significantly decreased MBN ($P<0.001$), increased MBC/MBN ($P<0.001$) and had no effect on MBC ($P>0.05$), respectively. N addition and clipping significantly decreased on average MBN by 17% ($P<0.001$) and 10% ($P<0.001$), respectively, while N addition increased MBC/MBN by 15% ($P<0.001$). For interactions between patch, sampling date, clipping and N addition see Table 2.

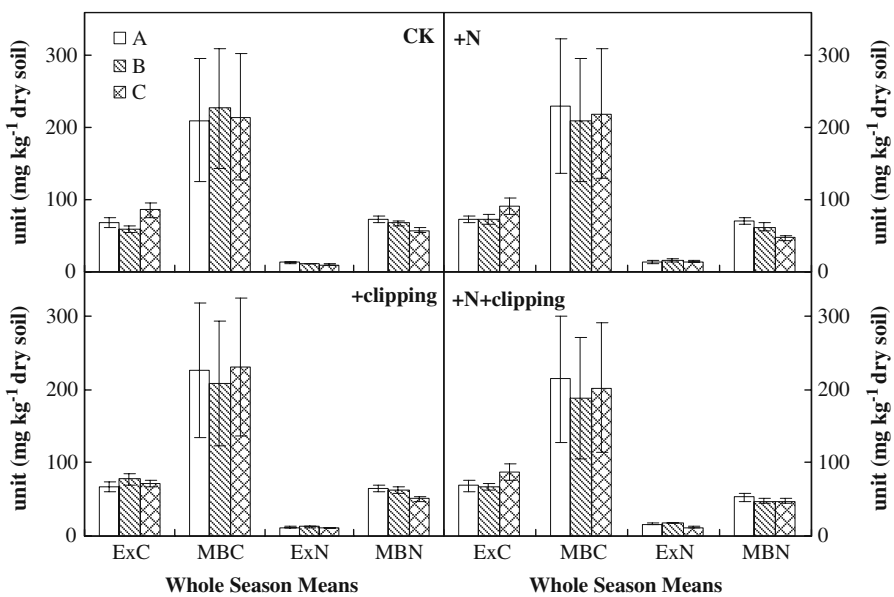

Fig. 5 The effects of clipping and N addition on extractable C (ExC), microbial biomass carbon (MBC), extractable N (ExN) and microbial biomass nitrogen (MBN) in soils (0–10 cm) of different vegetation patches (A-grass, B-mixed (grass+herbs), C-herbs) of the mean value over whole growing season (mean±1 SE) in 2008

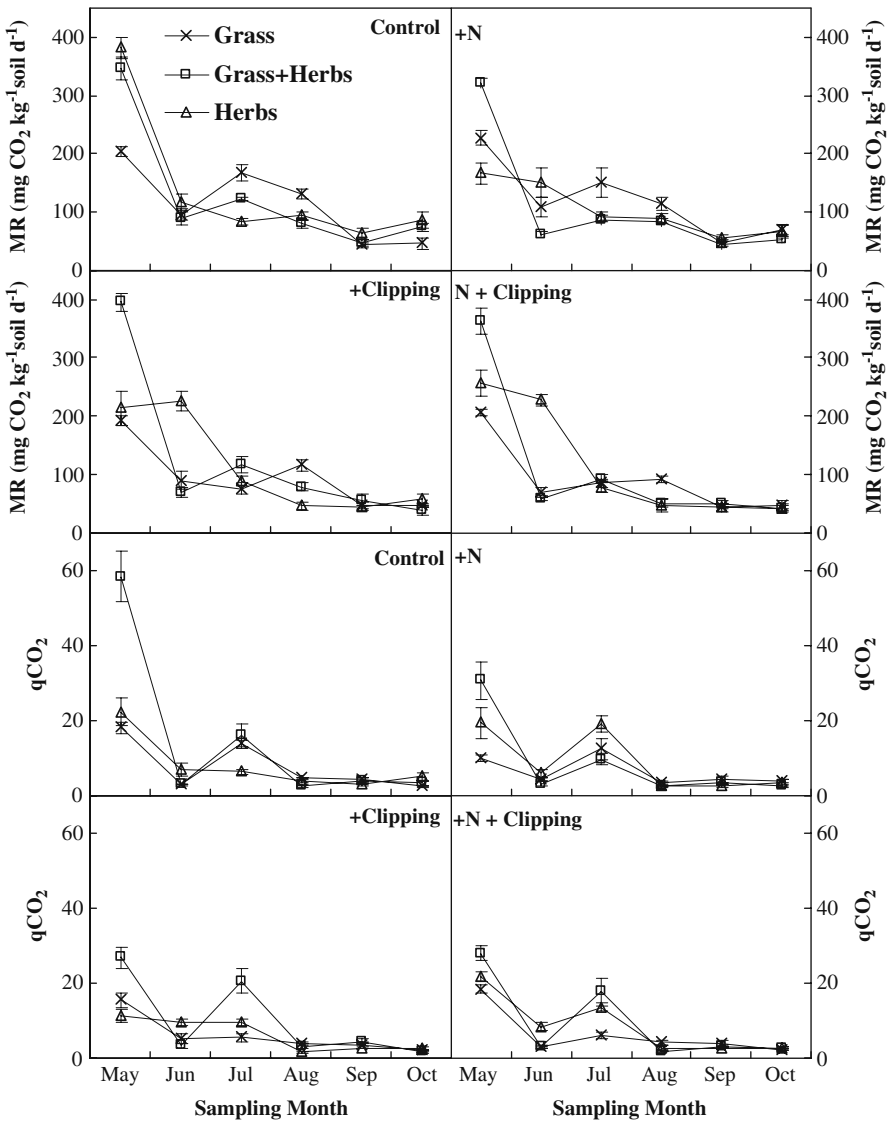

Fig. 6 The effects of clipping and N addition on microbial respiration (MR) and qCO2 in soils (0–10 cm) taken from different vegetation patches over the growing season (mean ±1 SE) in 2008

There were clear temporal trends in microbial respiration (MR) with peak values occurring in May (Fig. 6) under laboratory incubations. All four factors (patch, clipping, N addition and sampling date) were significantly affecting MR ($P<0.001$). MR was 12% higher in both mixed and herb patches as compared to grass patches ($P<0.001$). Clipping and N addition significantly decreased MR by 9% and 10% ($P<0.001$), respectively. In addition, sampling date and its interaction with patch, clipping, and N addition had significant impacts on MR ($P<0.001$, Table 2).

Vegetation patches also significantly affected the qCO_2, with values 61% and 34% ($P<0.001$) higher in mixed patches than in grass and herb dominated patches. Clipping decreased mean qCO_2 by 17% ($P<0.01$), whereas N addition had no effect on qCO_2 ($P>0.05$). For interactions between patch, N addition, clipping and sampling date on qCO_2 see Table 2

Discussions

Temporal and spatial variation in soil N transformation and microbial activity

Temporal variations in soil temperature and soil moisture can significantly drive microbial activities and N transformations in grassland ecosystems as

Makarov et al. (2003) have shown for alpine grassland in the Caucasus, Garcia and Rice (1994) for a prairie system in North America and Li and Chen (2004) for a large scale gradient study along a climate transect with different steppe types in Inner Mongolia. Also Wang et al. (2006) found in a laboratory study that N transformation and microbial activity in typical temperate steppe near our old field community field sites were driven by changes in soil temperature and moisture. On the other hand, other studies such as the one by Uri et al. (2003) on microbial N turnover in soils from an abandoned farm-land in the southeastern part of Estonia failed to demonstrate an expected correlation between soil environmental conditions and net mineralization. Also in our study we could not demonstrate a significant correlation between in-situ measured net N mineralization and observed monthly mean values of soil temperature and moisture. Such a lack in correlation can be due to insufficient variations in temperature and moisture. In consequence two interpretations are possible:

a) neither temperature nor soil moisture are being finally limiting for net mineralization or
b) net N mineralization was constantly limited by either soil moisture or soil temperature. Further below we will demonstrate that throughout our study period soil moisture was indeed limiting net N mineralization.

Other factors such as substrate quality have been found to be the reason for the lack of correlation between in situ net mineralization and soil moisture and temperature. Nadelhoffer et al. (1991) found that variation in substrate quality affected mineralization of organic matter in tundra soils more than variations in temperature. Also Binkley and Valentine (1991) reported that site differences in tundra soils were of higher importance for variations in net N mineralization than temperature and moisture changes.

Temporal variation in plant growth and soil C and N availability could also affect microbial N transformation (Xu et al. 2007) and microbial activities (Liu et al. 2007). Results from our experiment showed that the lowest rates of net N mineralization and nitrification occurred in August, i.e. during the period with peak biomass development (Fig. 4). Various mechanisms may have contributed to the coincidence of low net N mineralization and nitrification rates and peak biomass in August: the input of high C:N ratio substrates from root turnover and aboveground litter from senescence, coupled with high soil water availability, might have promoted net N immobilization during this period. This explanation is supported by findings of others who showed that input of labile C with high C/N ratio can stimulate rapid microbial growth and thus the assimilation and immobilization of mineral N into microbial biomass (Baggs et al. 2000; Manzoni et al. 2010).

The observed spatial variations in soil N transformation and microbial biomass at our field site in northern China were mainly due to differences in floristic composition. This observation is in agreement with results by Burke et al. (1999) and Hook and Burke (2000) who also found that plant species composition of abandoned arable land in the Central Plains of the USA affected microbial parameters. Burke et al. (1999) demonstrated that soil microclimate, plant growth, litter quality and belowground C and N inputs were driving N transformations and microbial biomass in two plant communities. They found that potentially mineralizable N and soil particle distribution differed between herb (O. *polyacantha*) and grass (B. *gracilis*) dominated vegetation patches with greater potential N mineralization and greater sand content (and lower clay and silt) under O. *polyacantha* than B. *gracilis*. In our study in situ N mineralization rates were lower in the patches dominated by grasses (Patch A) than in those dominated by herb (Patch C) (Online Table 2). The ratio of inorganic to total N was higher in soils under herb than under grasses, which was consistent with higher rates of net N mineralization and extractable C. On the other hand, with lower leaf N content, the C:N ratio was higher in herb dominated patches (leaf N concentration, 1.71%; leaf C:N, 27.6) as compared to the grass dominated patches (leaf N concentration, 2.09%; leaf C:N, 20.6; Wang et al., unpublished data). One would have expected that N mineralization is higher for sites with narrower litter C:N ratios (Zak et al. 2003), but this is obviously not the case in our study. This contradiction may be explained by general higher soil moisture values in the herb patches with less litter as compared to the grass dominated patches (Fig. 2) and by the higher lignin content of grass litter as compared to herb litter, which is known to negatively affect N mineralization (Vinton and Burke 1995; Arjan et al. 2010). Epstein et al (1998) reports that net N mineralization was significantly greater in

C-3 plots than in C-4 plots in a shortgrass steppe. In our experimental plots, the grass patches were dominated by a C-4 grass, and the herb patches by C-3 type plants, which may help to explain why net N mineralization rates were higher in herb patches than those in grass patches. C-4 plants have a higher water use efficiency and photosynthetic ability under environmental conditions of low water availability than is the case for C-3 plants. Also C-3 plants have lower levels of fiber and toughness as compared to C-4 plants (Barbehenn et al. 2004).

The ratio between in-situ measured field N mineralization rates and those measured under controlled conditions in the laboratory was calculated. Significant differences were observed among the three patches, and the ratio decreased in the order mixed> herb>grass. Thus, there was a tendency for field net N mineralization rates to be closer to potential net N mineralization rates for the mixed and the herb patches. This is consistent with the idea that environmental limitation of N mineralization by soil water was highest in the grass dominated patches and somewhat lower in the mixed and herb patches. This interpretation is supported by our measurements of soil water, which indicated that differences in water content among the three patches were large relative to the water holding capacity of the soil sampled.

The effects of clipping on N transformation and microbial activity

In our laboratory experiments we showed that clipping significantly increased ammonification rates (and the sum of ammonification+nitrification= mineralization) but did not affect nitrification rates. However, we failed to demonstrate a stimulatory effect of clipping on net N turnover rates by in situ incubations. Plant species and cover could have direct impacts on soil N transformation for different aboveground litter qualities as indicated by C:N ratios and labile C concentrations. Uhlířová et al (2005) demonstrated that the removal of plant material with a high C:N ratio is likely to facilitate rapid N cycling by limiting carbon input to the soil, thus maintaining rapid N turnover in the microbial community in the central mountain plateau of Bohemian Forest National Park, Czech Republic. In our study we found that clipping enhanced plant growth by 9.4%. Reasons for this observation can be increased light availability following clipping which may have stimulated plant growth in the early growing season (Hamilton and Frank 2001). On the other hand, our results also indicated that clipping reduced the availability of decomposable substrates (extractable C and N decreased 4.2% and 7.3% after clipping, respectively) for soil microorganisms. This observation is in agreement with results of Holland and Detling (1990) who found that grazing decreased root carbon inputs and in consequence limited carbon availability for decomposers.

Our results on MBC and MBN are in contrast to a study carried out in mesic grassland, in the central mountain plateau of Bohemian Forest National Park, Czech Republic, where clipping enhanced soil microbial biomass (Uhlířová et al. 2005). In our study we found that clipping decreased MBC and MBN by 10.3% and 7.1%, respectively. The different results between the Uhlířová et al. (2005) study and our study are possibly due to the vegetation type as well as soil characteristics, such as pH value which is lower (4.67) than our study site (6.84) in Northern China.

Soil microbial respiration was not sensitive to clipping in our experiments. However, if one calculates the qCO_2 value, i.e. the quotient of microbial respiration and microbial biomass carbon, one can show that following clipping the qCO_2 decreased by 16.2%. Clipping stimulates rhizodeposition, which stimulates nutrient turnover in the rhizophere, which means greater nutrient availability and uptake and greater growth (Bardgett et al. 1998; Hamilton and Frank 2001). As a consequence more easily decomposable substrate is available, which in turn led to increased utilization efficiency and a reduced metabolic quotient.

The effects of N addition on microbial activity

The relationship between plant nitrogen use and microbial-mediated nitrogen transformation from plant detritus has been reported for a variety of ecosystems such as an old-growth forest and other ecosystems (Hart and Stark 1997; Waldrop et al, 2004; Knorr et al, 2005). We examined the response of microbial N transformation and biomass to N fertilization in grass, mixed and herb dominated patches. Our results showed that N fertilization reduced microbial biomass nitrogen in all three patches significantly. Moreover, microbial respiration (mixed and herb patches) as well as net N mineralization

(mixed patch) was significantly reduced (Supplementary Table 2). These results are inconsistent with Fisk and Fahey (2001), who reported that soil microorganisms immobilized a higher proportion of mineralized N in fertilized plots after 8 years of N fertilization, and that this was likely due to greater specific activity and turnover of microbial biomass in fertilized plots. In agreement with results of our study Fisk and Fahey (2001) showed that microbial biomass and activity was significantly suppressed by mid- to long-term (>1 year) addition of nitrogen to hardwood. The reduction in the availability of N to microbes that occurs from N addition is likely a result of the reduced root allocation (Lee et al. 2010) and decreased flow of root exudates C. Such an interpretation is in-line with findings by Ajwa et al. (1999) who showed a reduction in root biomass by high N input and consequently lower soil microbial biomass. Lovell et al. (1995) found the same trends, i.e. that soil microbial biomass decreased more substantially under large N loads and longer durations of fertilization due to the synthesis of recalcitrant and toxic compounds under N addition. In our study, the question still remains as to why microbial activity and biomass would be reduced in response to N addition. It is very difficult to attribute all of the effects seen in this study solely to nitrogen availability. N addition not only increases levels of N in the soil but also can decrease soil pH, which is the reason for the reduction in microbial biomass, as was also observed in our study (Supplementary Table 1). Altogether, these results suggest that N enrichment could reduce microbial biomass in many ecosystems (Treseder 2008). In contrast, microbial biomass has been found to increase in response to shorter-term fertilization (Hart and Stark 1997; Supplementary Table 2). Garcia and Rice (1994) and Ding and Cai (2007) also observed N addition to increase soil microbial biomass nitrogen and to decrease microbial biomass carbon in a tallgrass prairie, possibly by modifying the composition of the microbial population. Other studies found no changes in soil microbial biomass or activities during the first growing season after N addition (Stark and Kytoviita 2006; Liu et al. 2007). The results from our experiment might be affected by the long-term N addition (3 years) at a rather high rate of 10 gNm^{-2} yr^{-1}. As shown by Fisk and Fahey (2001) the abundance and activity of soil microorganisms are regulated on the long-term by the availability of nitrogen.

Conclusions

The rates of microbial transformations varied significantly among the vegetation patches, which probably relates to direct effects of plant species on microbial N turnover rates as well as indirect effects via differences in soil moisture and temperature. Ammonification and net N mineralization rates were highest in the herb patches. Common management practices such as clipping and N addition could be shown to negatively affect microbial respiration in the old-fields investigated here, even though our understanding of the wider interaction with plant community structure and C and N cycling in soils in old-field communities is still limited. Plant association with the environment, rather than control over it, cannot be ruled out. However, such information is needed for developing adequate management strategies for maintaining soil fertility and principal soil functions such as nutrient retention. From our results, we can tell the land manager that herb species patch can get better nutrient cycling.

Acknowledgements This research was financially supported by the State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences and the Ministry of Science and Technology of China (2007CB106801). We wish to extend our thanks to the staff of the Duolun Restoration Ecology Experimentation and Demonstration Station. We are also grateful to Jianyang Xia, Zhe Zhang, Haijun Yang, and Delu Lin for their assistance in collecting samples and to Feng Zhu, Yanbo Yu, and Lina Niu for help in laboratory measurements. We also thank two anonymous reviewers of the original manuscript for constructive comments.

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