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RESEARCH ARTICLE

Contrasting effects of N fertilization and mowing on ecosystem multifunctionality in a meadow steppe

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ABSTRACT

There is little experimental field evidence on how multiple essential land use intensification drivers (LUIDs), such as nitrogen (N) fertilization and mowing, interact to control ecosystem multifunctionality. Here, we conducted a 4-year field experiment in a meadow steppe in northeast China and evaluated the direct and indirect effects of mowing and N fertilization on a range of ecosystem functions associated with nutrient cycle, carbon stocks, and organic matter decomposition during the past 2 years of the experiment (2017 and 2018). Mowing had negative effects on the ecosystem multifunctionality index (EMF), carbon (C) cycle multifunctionality index (CCMF), and N cycle multifunctionality index (NCMF) in 2 years of sampling. However, in general, the responses of multifunctionality to N fertilization were rate-specific and year-dependent. N fertilization had positive effects on EMF, CCMF, NCMF, and phosphorus (P) cycle multifunctionality index (PCMF) in 2017, with the higher precipitation rate during the growing season, which was likely associated with the strong monsoon season. However, in 2018, EMF, CCMF, and NCMF increased at the lower N fertilization levels ($\leqslant$ 10 g N m^{-2} yr^{-1}), but decreased at higher N rates. N fertilization had consistent positive effects on PCMF in the 2 years of sampling. The effects of land use drivers on multifunctionality were indirectly influenced by bacterial biomass, plant richness, and soil moisture changes. Our results also indicated that the impacts of land use drivers on multifunctionality played an important role in maintaining a range of functions at low levels of functioning (< 50% functional threshold). Low N fertilization levels ($\leqslant$ 10 g N m^{-2} yr^{-1}) were able to reduce the negative effects of mowing on ecosystem multifunctionality while promoting plant biomass (food for livestock) and C storage. These findings are useful for designing practical strategies toward promoting multifunctionality by managing multiple LUIDs in a meadow steppe.

1 Introduction

Land use intensification drivers (LUIDs), such as nitrogen (N) fertilization and mowing, influence various terrestrial ecosystem processes, including plant productivity, biodiversity stability, and nutrient cycles (Collins et al., 1998; Sala et al.,

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2000; Yang et al., 2012; Thébault et al., 2014; Gossner et al., 2016), and may contribute to serious degradation and desertification globally. Previous studies of land use intensification primarily focused on a single driver, particularly N fertilization (Reich et al., 2001; Manning et al., 2006; Fierer et al., 2012; Bradford et al., 2014), with only a few considering how multiple LUIDs (e.g., N fertilization and mowing) affect multiple ecosystem functions (multifunctionality) either on their own or by interacting with other drivers. However, more recently, new studies explored the combined effects of several LUIDs on multifunctionality (Blüthgen et al., 2012; Allan et al., 2015; Giling et al., 2019). Even so, there is still relatively little experimental field evidence that supports the link between land use intensification and multifunctionality in grasslands. Multiple LUIDs are known to simultaneously occur in terrestrial ecosystems worldwide, having immediate effects on multifunctionality. Therefore, we need an improved understanding of how individual or interacting LUIDs affect ecosystem multifunctionality to help make improved predictions of how the services and functions of a given ecosystem may change in an overpopulated and intensively managed world.

Grasslands are a good example of an ecosystem where multiple LUIDs simultaneously occur. For example, mowing is among the most common management strategies in grasslands, which aims to increase livestock food availability during winter. Extensive meadow steppe areas in northeast China, which are part of the largest remaining Eurasian grasslands worldwide, are mowed. These grasslands also receive large quantities of N through fertilizer application, from animal droppings, and by atmospheric deposition. Chinese grasslands have received more than 1.5 g N m^{-1} over the past 30 years (Liu et al., 2013). It is unclear how N fertilization and mowing jointly affect multifunctionality in these managed ecosystems, as evidences suggest that they both can individually alter various terrestrial ecosystem functions (e.g., plant biomass production; Bernhardt-Römermann et al., 2011). We hypothesized that mowing and N fertilization may have contrasting effects on ecosystem multifunctionality. For example, mowing is known to reduce atmospheric C inputs, which means fewer resources are available for soil microbes and less C is stored in plant biomass (Callaham et al., 2002). Conversely, N fertilization increases N availability in the soil and provides more resources for plants and microbes. Land that is subject to both mowing and N fertilization may suffer from nutrient imbalances, result in contrasting effects on multifunctionality, compared with untreated land or land that is either mowed or only N-fertilized. Several evidences show that the effects of LUIDs on ecosystem multifunctionality are indirectly regulated by soil property changes (e.g., pH and soil moisture), plant and microbial biodiversity, and bacterial and fungal biomass (De Vries et al., 2012; Delgado-Baquerizo et al., 2017a, b; Maestre et al., 2012; Wang et al., 2019, 2020). Therefore, we need to understand how mowing and N fertilization individually and interactively affect ecosystem multifunctionality and how ecosystem multifunctionality responds to diverse management scenarios. These will provide guidance for land managers and policy makers in formulating regulations that maintain ecosystem sustainability and human well-being under global change scenarios.

Here, we investigated how mowing and N fertilization individually and interactively affected ecosystem multifunctionality via structural equation modeling to identify the indirect (e.g., changes in soil properties, microbial biomass, and plant diversity) and direct effects of land use intensification on multiple ecosystem functions. We conducted a 4-year field study in a meadow steppe in northeast China to evaluate the overall effects of two LUIDs, namely simulated N fertilization (0, 2.5, 5, 10, 20, and 40 g N m^{-2} yr^{-1}; N0, N2.5, N5, N10, N20, and N40) and mowing, on ecosystem multifunctionality. Multifunctionality refers to multiple ecosystem functions associated with plant production, C storage, and enzyme activities, which are essential for sustainable managed ecosystem functioning by promoting food for livestock grazers and supporting key ecosystem services, such as C sequestration or organic matter decomposition (Table 1). Our study aims to provide experimental insights into the roles of mowing and N fertilization in driving ecosystem multifunctionality, and help land managers to find an equilibrium in maintaining plant production for grazers and promoting other important ecosys-

Table 1 The biogeochemical cycles, group of functions, full names, abbreviations, and services supported by the eleven ecosystem functions.

Biogeochemical cycle	Group of functions	Full name	Abbreviation	Service
C cycle	Plant production	Aboveground biomass	AGB	Providing food for livestock grazers, supporting food for humans
		Belowground biomass	BGB	
	Starch degradation	α-1,4-glucosidase	αG	Organic matter decomposition
	Starch degradation	β-1,4-glucosidase	βG	
	Hemicellulose degradation	β-1,4-xylosidase	βX	
	Cellulose degradation	β-D-cellobiohydrolase	CBH	
	Soil C stocks	Soil total organic carbon	TOC	Climate regulation
N cycle	Protein degradation	Leucine amino peptidase	LAP	Organic matter decomposition
	Chitin degradation	β-1,4-N-acetyl-glucosaminidase	NAG	
P cycle	Organic P mineralization	Alkaline phosphatase	ALP	Organic matter decomposition
	P availability	Available phosphorus	Available P	Nutrient cycling

tem services. Note that we did not include nutrient stocks as an ecosystem function for two reasons. First, nutrient stock is directly related to our treatment (e.g., N fertilization), therefore the related results could be difficult to interpret. Moreover, nutrient availability is often positively associated with ecosystem functions in natural ecosystems, whereas in managed ecosystems, eutrophication can be negative. Therefore, nutrient is not a positive multifunctionality contributor. The plant richness, soil properties (pH and soil moisture), and microbial biomass (bacterial, fungal, and arbuscular mycorrhizal fungal biomass) were also measured to assess their potential in regulating the effects of LUIDs on ecosystem multifunctionality.

2 Materials and methods

2.1 Study site

The study was conducted on a meadow steppe at the Songnen Grassland Ecology Research Station of Northeast Normal University, which is in the western Jilin Province, northeast China (44°45′ N, 123°45′ E). The study site elevation is between 152 and 172 m above sea level. Meadow steppe is a major component of the Eurasian steppe, one of the largest remaining grasslands worldwide. The study site has a temperate, semiarid, continental monsoon climate, with a mean annual temperature of 6.4°C (1950–2004) and mean annual precipitation of 471 mm (1950–2004), and more than half of rainfall occurred from June to August. Its soil type is chernozem meadow soil, which has a pH ranging from 8.0 to 10.0. The soil nutrient contents are low, with organic C and total N contents of approximately 2.0% and 0.15%, respectively (Cui et al., 2015). The grassland vegetation is dominated by *Leymus chinensis*, a C_3 perennial rhizomatous species commonly found in the eastern Eurasian steppes. Other species include *Phragmites australis*, *Chloris virgata,* and *Kalimeris integrifolia* (Wang et al., 2019).

2.2 Experimental design

The experimental area (200 m × 100 m) was initially fenced to exclude livestock grazing (cattle and sheep) in 2010. Then, we established eight blocks with similar vegetation (each 35 m × 35 m) within the fenced area in May 2015, with a buffer zone of at least 2 m between the blocks. Each block was divided into 36 plots (each 5 m × 5 m) within each block, with a buffer zone of at least 1 m between the plots. Each plot was randomly assigned with N treatment (0, 2.5, 5, 10, 20, or 40 g N m^{-2} yr^{-1}), P treatment (0, 5, or 10 g P m^{-2} yr^{-1}), mowing treatment (mown or unmown), or a random combination of N, P, and mowing treatments (shown in Fig. S1), which had excessive fertilization input due to atmospheric nutrient deposition in northern China (10 g N m^{-2} yr^{-1} and 5 g P m^{-2} yr^{-1}; He et al., 2007; Yang et al., 2012), but conformed to the actual situation in the Chinese agricultural-pastoral zone. The selected treatments in the present study were N fertilization (0, 2.5, 5, 10, 20, or 40 g N m^{-2} yr^{-1}), mowing (mown or unmown), and both random combinations of N fertilization (six concentrations) and mowing (absence and presence). The solution used for N fertilization comprised a 7:3 mixture of inorganic (NH_4NO_3) and organic (urea) N. The total annual N application was divided equally into five parts and applied once a month from May to September of each year from 2015 to 2018. N was dissolved in 2.5 L purified water and sprayed onto each treatment plot, as well as onto the control plots. The plots were mowed with a hay mower in early August each year from 2015 to 2018, and the mowed plants were immediately removed from the plots and excluded from the aboveground biomass calculation. We chose early August for removing the aboveground plants by mowing, because it is the normal harvest time for the grasslands in north China. The precipitation data were collected using a weather station (HOBO U30-NRC, Onset, USA) installed in the experimental area for 2 years (from 1 September 2016 to 31 August 2018) to assess how precipitation affects grassland functions.

2.3 Soil sample collection and analysis

A five-point method was used to collect five soil samples at a depth of 0–10 cm with a 2.5-cm diameter soil auger from each plot in August 2017 and 2018. The samples were thoroughly mixed and immediately sieved (2-mm mesh) to remove shells, roots, and stones. Then, the soil samples were divided in the laboratory and analyzed for several properties. Soil hydrolytic enzyme activity and bacterial and arbuscular mycorrhizal fungi (AMF) biomasses were determined using a soil aliquot that was stored at –80°C and freeze-dried using a Labconco freeze-drier (Labconco, Kansas City, MO, USA) within 2 weeks after sampling. Available P was determined using another aliquot of soil that was stored at –20°C. Total organic C and pH were measured using a third aliquot of soil that was air-dried at room temperature in the laboratory. For pH, the air-dried soil was suspended in deionized water (soil:water = 1:5, w:v) and measured using a PHS-3E glass pH electrode (Leichi, Shanghai, China). Soil moisture content (SMC) was taken as the weight lost by oven-drying fresh soil at 105°C for 24 h or until the weight remained constant. The total C (TC) and N (TN) levels were determined using an element analyzer (vario EL cube, Elementar, Langenselbold, Germany). Microbial biomass C (MBC), N (MBN), and P (MBP) were measured using chloroform fumigation-extraction methods (Vance et al., 1987). Organic C and N in the solution were determined using a total organic carbon (TOC) analyzer (Vario TOC, Elementar, Germany). Phosphate was measured through the molybdenum blue colorimetric method using UV photometry at 880 nm.

2.4 Plant species richness and different microbial community biomasses

Three survey quadrats (0.5 m × 0.5 m) were fenced off and established in each plot in May 2015. Total plant species was counted in each plot in early August 2017 and 2018. The bacterial, fungal, and AMF biomasses in the pooled soils collected using the five-point method in each plot were determined using the phospholipid fatty acid method (Frostegård et al., 2011). The different C chain varieties that represented soil microbe species were analyzed using a DB-5 column in a gas chromatography–mass spectrometry (GC–MS) system (Thermo TRACE GC Ultra ISQ, Thermo Scientific, Walter, MA, USA). We used 15:0, i15:0, a15:0, i16:0, 16:1ω7, 16:1ω9, i17:0, a17:0, 17:0, i19:0, 18:1ω7, and 18:1ω5 as bacterial community indicators (Frostegård and Bååth, 1996); 18:2ω6,9 (Olsson et al., 1995; Frostegård and Bååth, 1996) and 18:1ω9c (Bååth and Anderson, 2003) as fungal community indicators, and 16:1ω5 as an AMF indicator (Olsson et al., 1995).

2.5 Measures of ecosystem functions

2.5.1 Plant production, soil TOC, and available P

We determined the plant aboveground and belowground biomass (AGB and BGB) through the harvest method in August 2017 and 2018 before mowing. The plant AGB in each plot was harvested from a randomly placed quadrat (0.5 m × 0.5 m). We sampled the BGB in the same quadrat by washing the roots from a soil core collected at 30 cm depth using a 10-cm diameter soil auger. We oven-dried the plant samples at 65°C for 48 h until the weight of the plant materials were constant. The soil samples were treated with 1M HCl to remove total inorganic C, and soil TOC was determined using an element analyzer (vario EL cube, Elementar, Langenselbold, Germany). Soil available P was measured through the molybdenum blue colorimetric method using UV spectrophotometry at 880 nm (Vance et al., 1987).

2.5.2 Soil hydrolytic enzyme activities

The activities of seven enzymes associated with C, N, and P cycle indices were measured using the fluorescent substrate labeling method described by Bååth and Anderson (2003) and Trivedi et al., (2016). For C cycle related enzyme measurement, substrate 4-MUB-α-D-glucopyranoside was used for α-1,4-glucosidase (αG) measurement, 4-MUB-β-D-glucopyranoside for β-1,4-glucosidase (βG), 4-MUB-β-D-xylopyranoside for β-1,4-xylosidase (βX), and 4-MUB-β-D-cellobioside for β-D-cellobiohydrolase (CBH); whereas for N cycle related enzyme measurement, substrate L-leucine-7-amino-4-methylcoumar was used for leucine amino peptidase (LAP) and 4-MUB-N-acetyl-β-D-glucosaminide for β-1,4-N-acetyl-glucosaminidase (NAG) measurements. Also, 4-methylumbelliferyl phosphate was used to measure alkaline phosphatase (ALP), an indicator of the P cycle index. Fluorescence values were determined using a fluorescence plate reader (TECAN infinite F200, Tecan Group, Switzerland) with 360 and 460 nm excitation and emission filters, respectively. The following equations were used to calculate the enzymatic activities:

$$\text{Activity}(\text{nmol g}^{-1}\,\text{h}^{-1}) = \frac{\text{Net fluorescence} \times 100\,\text{mL}}{\text{Emission coefficient} \times 0.2\,\text{mL} \times 3\,\text{h(Time)} \times 1\text{g(Soil weight)}} \tag{1}$$

where,

$$\text{Net fluorescence} = \left(\frac{\text{Sample assay} - \text{Soil control}}{\text{Quench coefficient}}\right) - \text{Negative control} \tag{2}$$

$$\text{Emission fluorescence}(\text{fluorescence. nmol}^{-1}) = \frac{\text{Reference standard}}{0.5\,\text{nmol}} \tag{3}$$

$$\text{Quench coefficient} = \frac{(\text{Quench standard} - \text{Soil control})}{\text{Reference standard}} \tag{4}$$

2.6 Assessing ecosystem, C, N and P cycle multifunctionality

We evaluated ecosystem multifunctionality using 11 ecosystem functions that provide many fundamental services for people, including AGB, BGB, αG, βG, βX, CBH, TOC, LAP, NAG, ALP, and available P (Table 1). To determine the average multifunctionality, we calculated *Z*-scores (standard deviations) of the 11 functions evaluated before analysis (Maestre et al., 2012), and considered the ecosystem multifunctionality index (EMF) as the average *Z*-score of all the 11 functions measured within a treatment, whereas C cycle multifunctionality index (CCMF) is the average *Z*-score of AGB, BGB, αG, βG, βX, CBH, and TOC. The N cycle multifunctionality (NCMF) is the average *Z*-score of LAP and NAG, and the P cycle multifunctionality (PCMF) is the average *Z*-score of ALP and available P. The weighed EMF was calculated as previously described by Manning et al. (2018). Moreover, we calculated the number of functions beyond different thresholds of 10%, 25%, 50%, 75%, and 90%, following the multi-threshold approach (Byrnes et al., 2014).

2.7 Statistical analyses

The individual or combined effects of N fertilization, mowing, and year on EMF, CCMF, NCMF, and PCMF were analyzed using a three-way ANOVA test and visualized using the box plot method. We tested the normal distribution using the Shapiro–Wilk test using the SPSS 23 software before all ANOVA analyses. The *varpart* function was used to partition the variance of the total explained variance caused by the effects of the two treatments (N fertilization and mowing), year, and environmental parameters (Env), namely precipitation, plant richness, soil pH, SMC, bacterial and fungal biomass, MBC, MBN, MBP, and soil C:N ratio. All of the statistical analyses and visualizations were performed using the *data sets*, *RColorBrewer*, *lavaan*, *vegan,* and *ggplot2* packages in the R v.3.5.3 software.

Structural equation modeling (SEM) (Grace, 2006) was conducted to determine the direct and indirect effects of LUIDs (N fertilization and mowing), year, and plant richness, as well as bacterial and AMF biomasses on EMF, CCMF, NCMF, and PCMF based on the hypothesis model showing the potential relationship between two variables (shown in Fig. S2). The results of the SEM were shown in Tables S1–10. The number of individual functions that operated beyond a given critical threshold (10%, 25%, 50%, 75%, and 90%) was calculated using the multi-threshold approach (Byrnes et al., 2014). The SEM results were run using the *lavaan* package in the R v.3.5.3 software. The models' "goodness of fit" was indicated using the χ^2 test ($p > 0.05$) and root mean square error of approximation (RMSEA; < 0.05), as previously described by Delgado-Baquerizo et al. (2016) and Wang et al. (2019). Principal component analysis (PCA) was used to reduce the dimensions and visualize the direction and strength of the ecosystem's individual functions or multi-functionality relative to the overall distribution. PCA was conducted using the *factoextra* package in the R v.3.5.3 software.

3 Results

3.1 Unraveling the effects of mowing and N fertilization on ecosystem multifunctionality

Our results showed that N fertilization and mowing had contrasting effects on ecosystem multifunctionality (Figs. 1, S3). In general, mowing had negative effects on EMF, CCMF, NCMF, and weighted EMF in 2 years of sampling (Figs. 1, S3). N fertilization had positive effects on EMF, CCMF, NCMF, PCMF, and weighted EMF along all N gradients in 2017 (Figs. 1 and S3a). However, N fertilization had negative relationships with EMF, CCMF, NCMF, and weighted EMF when the rates exceeded 10 g N m^{-2} yr^{-1} in 2018 (Figs. 1A–C, S3a). N fertilization had consistently positive effects on PCMF in 2 years of sampling (Fig. 1D). We also found that interactive effects between LUIDs and year on EMF, CCMF, NCMF, and weighted EMF indicated possible regulatory effects of inter-annual climate variations on the relationship between LUIDs and ecosystem functions (Figs. 1 and S3a). For instance, the

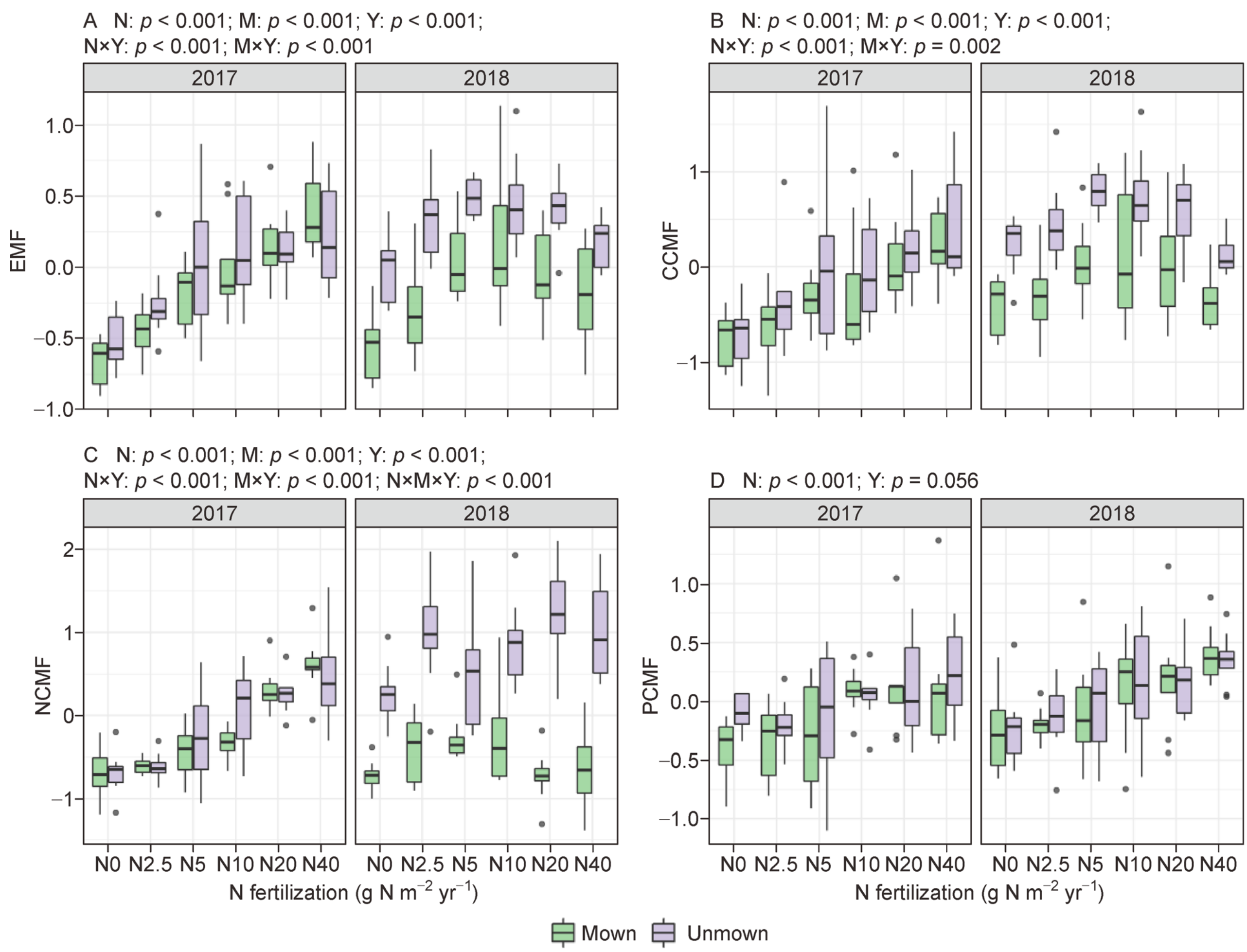

Fig. 1 Effects of N fertilization (N), mowing (M), year (Y), and their interactions on (A) EMF; (B) CCMF; (C) NCMF and (D) PCMF. Three-way ANOVA were used to test the significance of treatments and year. For clarity, only the significant statistical results ($p < 0.05$) are shown in the figure. EMF, ecosystem multifunctionality index; CCMF, C cycle multifunctionality index; NCMF, N cycle multifunctionality index; PCMF, P cycle multifunctionality index.

negative effects of mowing on EMF, CCMF, and NCMF were more noticeable in 2018 than in 2017 (Fig. 1A–C), which reflect that climate conditions regulated the responses of multifunctionality to N fertilization or mowing (drier in 2018 than in 2017; Fig. S4). The results indicated that annual sampling predicted how ecosystem functions responded to the changes in environmental conditions (e.g., soil moisture, Fig. S5f).

N fertilization and mowing had several interactive effects on NCMF. Thus, the effect of mowing on NCMF was highest under the largest N fertilization rate (Fig. 1C). Unlike CCMF and NCMF, several ecosystem functions associated with the P cycle were highly resistant to mowing, and no significant effect of mowing on PCMF was observed (Fig. 1D). However, N fertilization had a positive effect on PCMF by increasing phosphatase activity (Fig. 1D).

3.2 Responses of individual ecosystem functions and attributes to mowing and N fertilization

Land use intensification affected several important ecosystem attributes. For example, plant richness, fungal and AMF biomass, and soil pH were negatively affected by increasing N fertilization; however, mowing had positive effects on plant richness and microbial biomass (bacterial, fungal, and AMF communities, Fig. S5). Mowing also had a positive effect on soil pH and a negative effect on moisture content, and the values of soil moisture were lower in 2018, compared with those in 2017 (Figs. S5e, f). Interactions also existed between mowing and N fertilization for some ecosystem attributes. For example, the bacterial biomass increased under mowing treatment when the N fertilization rates were low, but decreased when N application rates exceeded 10 g N m^{-2} yr^{-1} (Fig. S5b).

Mowing and N fertilization had observable contrasting effects on most individual ecosystem functions. The results showed that specific functions have response patterns that could vary with LUIDs and year. The changes of the individual ecosystem functions were often greater in the second sampling year when the climate was dry (Figs. S6b, j). Interactions were present between LUIDs for several ecosystem functions. For instance, AGB had higher functional values in mowed areas when N fertilization rates exceeded 10 g N m^{-2} yr^{-1} (Fig. S6a). Several functions were only affected by one land use driver. For instance, TOC, phosphatase activity, and soil available P were only affected by N fertilization (Figs. S6i–k).

3.3 Responses of multi-dimensional and multi-threshold multifunctionality to mowing and N fertilization

PCA was performed to evaluate the multi-dimensional ecosystem multifunctionality for the 11 selected individual functions. The first two principal axes, PC1 (38.6%) and PC2 (15.1%), explained 53.7% of the total variances. Our multifunctionality index' multi-dimensional nature is shown in Fig. S7.

We found that mowing and N fertilization had strongly opposing effects on the number of functions over varying thresholds (10%, 25%, and 50%) when the multi-threshold multifunctionality approach was applied (Fig. 2). The individual effects of mowing and N fertilization on the number of functions at high thresholds (> 75% and 90%; $p > 0.05$) were weaker than the number of functions at low thresholds (Fig. 2), and the interactions were stronger at medium–high thresholds (> 50% and 75%; Fig. 2C, D). Furthermore, the variance proportion explained by the SEMs beyond the given thresholds strongly decreased from low threshold levels (> 20% of the explained variance; ⩽ 50%) to high threshold levels (< 5% of explained variance; > 75% and 90%; Fig. S8). The overall effects of mowing and N fertilization were weakened when the thresholds were > 75% (Fig. S8d, e; $p > 0.05$).

3.4 Overall direct and indirect responses of ecosystem multifunctionality to mowing and N fertilization

LUIDs generally explained unique portions of EMF, CCMF, NCMF, PCMF, weighted EMF, and individual function variations ($p < 0.05$; Figs. 3, S3b, S9). However, the effects of N fertilization and mowing on ecosystem functions were dependent on the specifically valuated function. Therefore, mowing explained more CCMF and NCMF variations (12% and 22%, respectively) than N fertilization did (Fig. 3B, C). Furthermore, N fertilization was most important to PCMF (Fig. 3D), explaining 18% of the variance. Environmental parameters explained 32% of the variance in EMF (Fig. 3A), which was highest among the LUIDs.

SEMs illustrated the overall direct and indirect responses of ecosystem multifunctionality and multi-threshold multifunctionality to individual LUIDs. Mowing had direct negative effects, but N fertilization and year had direct positive effects on EMF, CCMF, NCMF, and weighted EMF (Figs. 4A–C, S3c). In contrast, only N fertilization had significantly direct positive effects on PCMF (Fig. 4D). We also found that important ecosystem attributes, such as microbial biomass, soil moisture, and plant richness, mediated the indirect effects of land use drivers on ecosystem multifunctionality. The indirect effects of mowing on ecosystem functions were generally mediated by suppressing the positive effects of bacterial biomass on multifunctionality through the decreased soil moisture. N fertilization's positive effects on multifunctionality were indirectly influenced by decreased plant richness, thereby promoting bacterial community biomass (Fig. 4). N fertilization also promoted soil moisture and individual soil functions while increasing bacterial biomass (Figs. 4, S5, S6). Similar results were also found for the multi-threshold multifunctionality evaluation (Fig. S8).

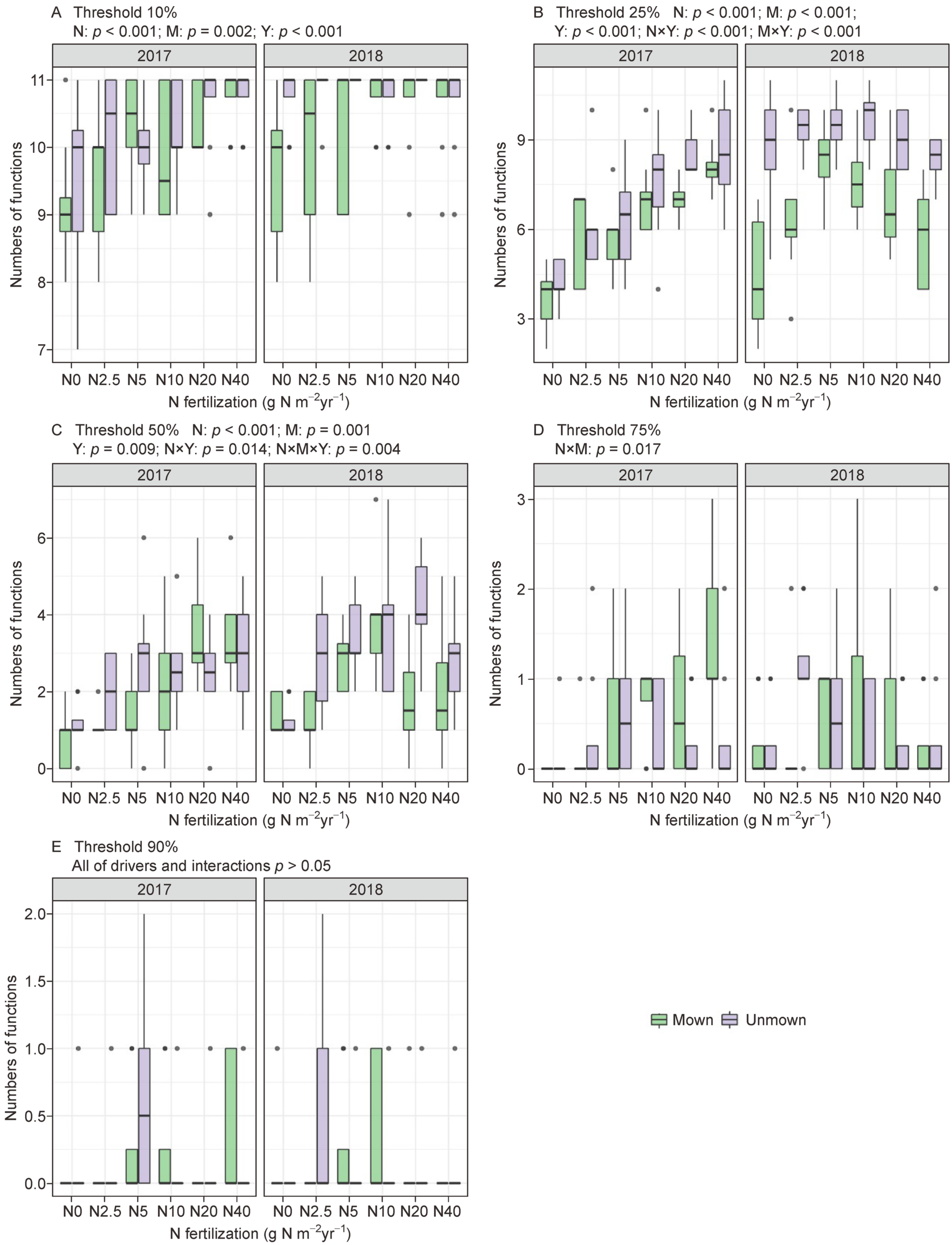

Fig. 2 Effects of N fertilization (N), mowing (M), year (Y), and their interactions on the numbers of functions above thresholds of (A) 10%, (B) 25%, (C) 50%, (D) 75%, and (E) 90%, calculated using the multi-threshold approach. Three-way ANOVA were used to test the significance of treatments and year. For clarity, only the significant statistical results ($p < 0.05$) are shown in the figure.

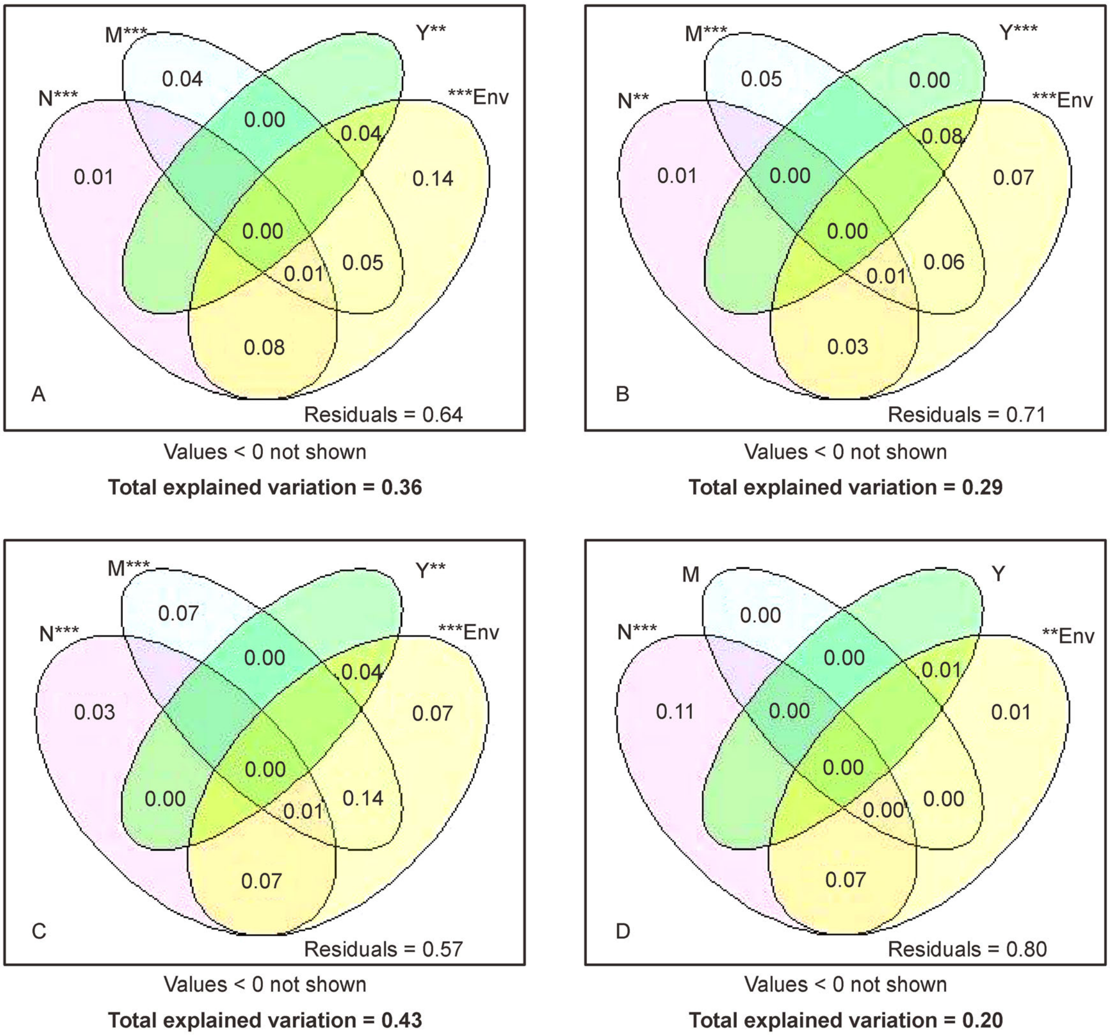

Fig. 3 Variance partitioning of the total explained variance of ecosystem multifunctionality using four independent groups of variables: N fertilization (N), mowing (M), year (Y), and environmental parameters (Env; including precipitation, plant richness, pH, soil moisture content, bacterial and fungal biomass, MBC, MBN, MBP and soil C:N ratio). (A) ecosystem multifunctionality index (EMF); (B) C cycle multifunctionality index (CCMF); (C) N cycle multifunctionality index (NCMF) and (D) P cycle multifunctionality index (PCMF). The residuals indicated the variances that were not explained by the explanatory variable. The significances of Monte Carlo permutation test (999 permutations) were shown as follows: *$p < 0.05$; **$p < 0.01$, ***$p < 0.001$.

4 Discussion

4.1 The responses of multifunctionality to N fertilization and mowing are dependent on the type of land use and functional thresholds

A combination of LUIDs (e.g., N fertilization, mowing, and grazing) can generally have direct and indirect effects on multiple functions across observed environmental gradients in terrestrial ecosystems (Laliberté et al., 2010; Allan et al., 2014, 2015), but there is few evidence on how multiple LUIDs simultaneously affect multifunctionality. Our results indicate that not all LUIDs negatively affect multifunctionality, and the independent effects of these drivers should be considered in understanding the responses of ecosystem functions under intensive management. In general, mowing had direct negative effects and N fertilization had direct positive effects on multifunctionality (Fig. 4). It does not necessarily mean that N supplementation to a field is beneficial for long-term multifunctionality maintenance, because N fertilization also has multiple cascading effects on the structure and function of terrestrial ecosystems (Galloway et al., 2003). For example, N fertilization promotes phosphatase activity, causing the increases in P cycle functions, resulting in soil P depletion and nutrient imbalances (Deng et al., 2016). N enrichment initially increases ecosystem productivity, but the positive effects decrease with long-term application, as it is indirectly driven by plant species depletion (Isbell et al., 2013). Together, these results indicate that future experiments should be done to elucidate multiple and contrasting effects

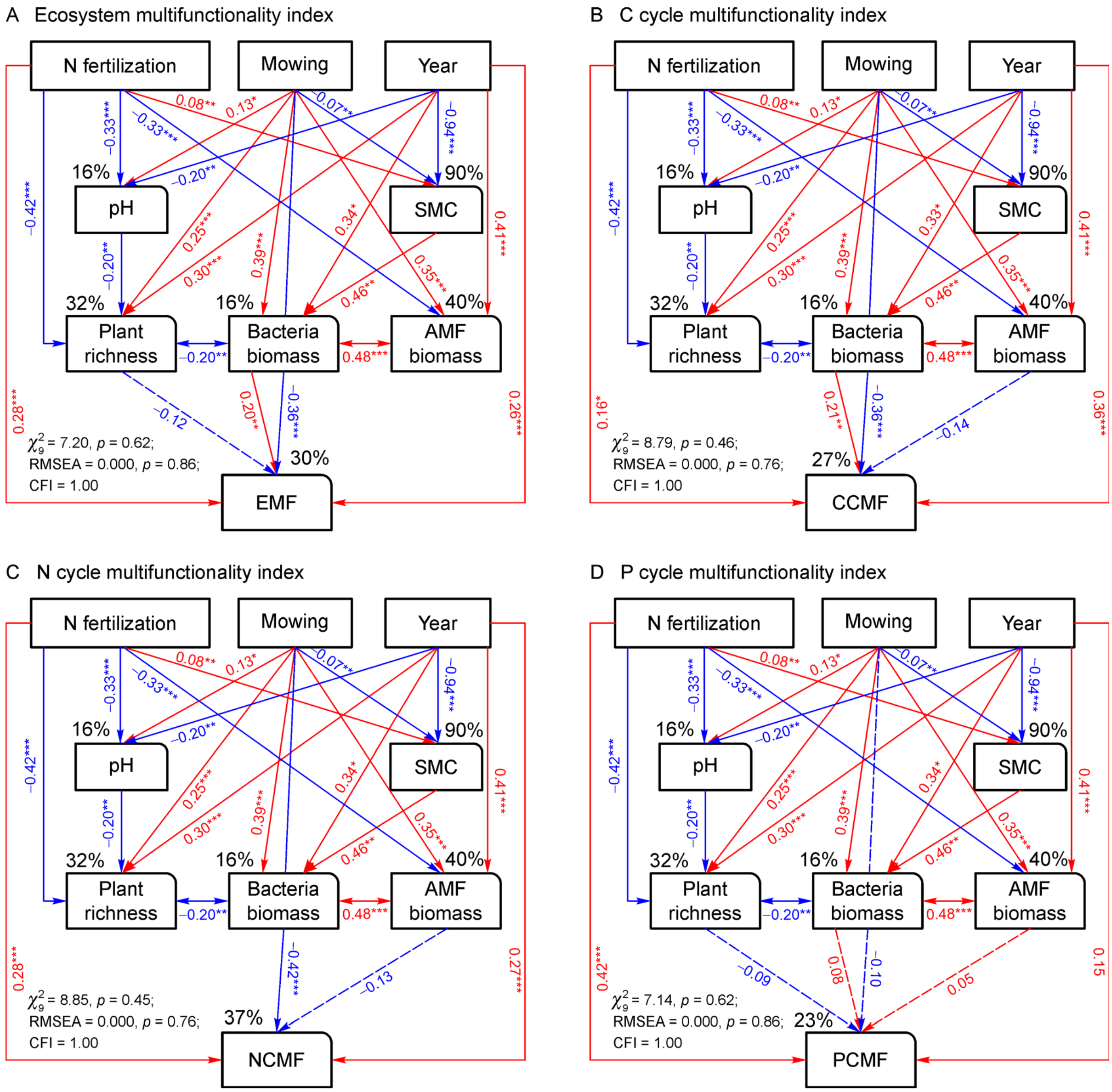

Fig. 4 Structural equation model (SEM) depicting the direct and indirect effects of N fertilization, mowing, year, and plant and soil attributes on multifunctionality. (A) EMF; (B) CCMF; (C) NCMF and (D) PCMF. The numbers on the arrows were the standardised path coefficients. The width of the arrows indicated the strength of the relationships. The red and blue arrows indicated significant positive and negative relationships, respectively ($p < 0.05$). The dashed lines indicated nonsignificant relationships ($p > 0.05$) (see Methods). Percentages close to the endogenous variables indicated the variance explained by the model (R^2). *$p < 0.05$, **$p < 0.01$; ***$p < 0.001$. pH, soil pH; SMC, soil moisture content; AMF biomass, arbuscular mycorrhizal fungal biomass; EMF, ecosystem multifunctionality index; CCMF, C cycle multifunctionality index; NCMF, N cycle multifunctionality index; PCMF, P cycle multifunctionality index.

of LUIDs on ecosystem functions, which may not be obvious from observational studies.

Interestingly, further analyses showed that the effects of N fertilization and mowing on ecosystem functions differed across contrasting functional thresholds, which agreed with previous studies (Byrnes et al., 2014; Wang et al., 2019). Therefore, we found strong direct and indirect effects of land use drivers on several individual functions beyond specific thresholds. Also, the extent of these effects varied across threshold levels, with weaker effects at high thresholds level (> 75% and 90%; Figs. 2, S8) than at low threshold levels, that is, most functions worked at low-level thresholds (< 50%). Therefore, only a few functions had crucial roles in driving multifunctionality at high threshold levels (> 75% and 90% thresholds). This interesting result suggests that N fertilization and mowing have greater influences on ecosystem functions at a relatively low-level threshold than at very high threshold levels (e.g., BGB and βG). These findings indicate that multiple functional thresholds should be considered when determining the effects of LUIDs on multiple ecosystem functions in managed terrestrial ecosystems.

4.2 The rate-specific responses of multifunctionality to N fertilization are year-dependent

This study provides novel evidence on how N fertilization affected ecosystem multifunctionality differently in 2 years with contrasting precipitation conditions (i.e., drier in 2018 than in 2017, Fig. S4). Our results indicate the interactive effects of N fertilization and interannual climatic variations (e. g., precipitation) on multiple ecosystem functions. This agreed with previous studies on meadow steppes where water availability and N addition exhibited synergistic effects on soil C sequestration (Bi et al., 2011; Niu et al., 2009). In line with this, Delgado-Baquerizo et al., (2017a) suggested that ecosystem multifunctionality is significantly impacted by water availability in drylands worldwide. N fertilization consistently increased multifunctionality along different gradients in 2017 (the year with high rainfall level), which is most likely associated with the strong monsoon season. However, in 2018, higher N levels (>10 g N m^{-2} yr^{-1}) decreased multiple ecosystem functions associated with plant production, C storage, and enzyme activities, whereas lower N fertilization rates (≤10 g N m^{-2} yr^{-1}) promoted these functions (Fig. 1). Excessive N input (e.g., 40 g N m^{-2} yr^{-1}, Fig. S5) significantly reduced bacterial and AMF biomass, which is also consistent with previous studies (Bai et al., 2010; Yao et al., 2014), indicating that the effects of N fertilization on ecosystem multifunctionality are rate-specific. Our results highlighted the importance of optimal N fertilization rate determination in promoting ecosystem multifunctionality for sustainable grassland management.

4.3 The shift of microbial biomass is a key mechanism regulating the effects of N fertilization and mowing on multifunctionality

Several effects of LUIDs on ecosystem multifunctionality were indirectly influenced by changes in biotic and abiotic factors, such as microbial community composition, soil pH and moisture (Delgado-Baquerizo et al., 2017a, b), and above- and belowground biodiversity (Maestre et al., 2012; Wang et al., 2019). Similar to previous studies (De Vries et al., 2012; Valencia et al. 2018), we observed that the opposite effects of LUIDs were indirectly driven by changes in microbial biomass regulated by soil pH, soil moisture, and plant richness (Fig. 4). For example, mowing had negative effects on soil moisture by indirectly decreasing the bacterial biomass, thereby decreasing ecosystem multifunctionality. Similarly, the positive effects of N fertilization on multifunctionality were also indirectly influenced by changes in bacterial biomass, which were regulated by plant richness and soil pH (Fig. 4). Our findings agreed with a recent report that plant diversity plays an important role in regulating soil multifunctionality responses to resource availability shifting (Yan, et al., 2020). In fact, the effects of plant richness on multifunctionality, as evaluated in this study, were affected by changes in microbial biomass. This is consistent with previous reports that suggested that the effects of plant diversity on ecosystem functions are mainly regulated by changes in microbial communities (Jing et al., 2015; Delgado-Baquerizo et al., 2016; Valencia et al., 2018). Yao et al. (2014) suggested that excessive N can alter soil pH, especially when N causes soil pH to drop below 6; hence, bacterial biomass and diversity significantly decrease in a steppe ecosystem, which finally decreases its multifunctionality, due to the importance of soil pH as a major driver of soil microbial communities (Fierer and Jackson, 2006) and multifunctionality (Delgado-Baquerizo et al., 2017a). However, we failed to detect the direct links between microbial biomass or multifunctionality and soil pH under land use intensification. This may be attributable to the originally high pH and minimal land use intensification decrease in the study site (Fig. S5e). Our study highlights the importance of microbial biomass in regulating how LUIDs (mowing and N fertilization) affect multifunctionality, given the positive association between multifunctionality and high microbial biomass in a warming experiment (Valencia et al., 2018).

5 Conclusion

This study demonstrated the contrasting effects of N fertilization and mowing on ecosystem multifunctionality in a meadow steppe. The positive effects of N fertilization were rate-specific and year-dependent, whereas mowing consistently decreased multifunctionality. The indirect effects of mowing and N fertilization were largely affected by changes in microbial biomass, which were positively associated with multifunctionality. Mowing led to soil moisture reduction, which in turn inhibited bacterial biomass' positive effects on multifunctionality. In contrast, plant richness decreased under N fertilization, as it was indirectly regulated by soil pH decrease, which promoted the positive associations between bacterial biomass and its associated functions. Our results also indicated that multiple functional thresholds should be considered when evaluating the effects of land use intensification on ecosystem functioning. N fertilization at the low rate of $\leqslant$ 10 g N m^{-2} yr^{-1} promoted important ecosystem functions that are critical for feeding livestock grazers (e.g., plant biomass) and other essential ecosystem services, such as organic matter decomposition and C storage. This study provides crucial application and guiding significance in maintaining multifunctionality through multiple LUID management in grassland ecosystems.

Authorship

H.C., W.S. and M.D-B. developed the original idea of the analyses presented in the manuscript. H.C., W.S. and J-Y.M. designed the field experiment. K.W., W.Z.S. and X.L. helped field and laboratory work by sampling plant and soil and analyzing all the functions. H.C. performed all the statistical analyses and modeling, and wrote the first draft supported by M.D-B. and W.S. All the authors contributed substantially to the revisions of the manuscript.

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Conflict of interest

The authors declare no conflicts of interest.

Electronic supplementary material

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