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REGULAR ARTICLE

Effects of seeding ratios and nitrogen fertilizer on ecosystem respiration of common vetch and oat on the Tibetan plateau

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Abstract
Background and aims Few studies have investigated the effect of nitrogen (N) fertilizer on ecosystem respiration (Re) under mixed legume and grass pastures sown at different seeding ratios,and data are almost entirely lacking for alpine meadow of the Tibetan Plateau. Our aim was to test the hypothesis that although a combination of legumes with grass and N fertilizer increases Re the combination decreases Re intensity (i.e. Re per unit of aboveground biomass) due to greater increases in aboveground biomass compared to increases in Re.
Methods This hypothesis was tested using different seeding ratios of common vetch (*Vicia sativa L.*) and oat (*Avena sativa L.*) with and without N fertilizer on the Tibetan plateau in 2009 and 2010. Re was measured using a static closed opaque chamber. Re intensity was estimated as the ratio of seasonal average Re during the growing season to aboveground biomass.
Results Compared with common vetch monoculture pasture, mixed legume-grass pastures only significantly decreased Re intensity (with a decrease of about 75 %–87 %) in the drought year 2009 due to greater increases in aboveground biomass compared to increases in Re. There were no significant differences in Re and Re intensity among different seeding ratios of oat and common vetch in either year. N fertilizer significantly decreased Re intensity for common vetch monoculture pasture by 24.5 % in 2009 and 69.5 % in 2010 although it did not significantly affect plant production and Re.
Conclusions From the perspective of forage yield and Re, planting mixed legume-grass pastures without N fertilizer is a preferable way to balance the twin objectives of forage production and mitigation of atmospheric greenhouse gas emissions in alpine regions.

Keywords Pasture · Mixed seeding · Monoculture · Ecosystem respiration · Respiration intensity · Alpine meadow

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Introduction

Management practices on agricultural lands, including nitrogen (N) fertilizer, changes in cropping system and tillage practices, have a significant impact on soil carbon (C) content (Ball et al. 1999; Baggs 2006; Omonode et al. 2007; Alluvione et al. 2009; Vanden Bygaart et al. 2003; Gregorich et al. 2005). As shifts in respiration can be the dominant control on interannual variation in the net C sink-source status of an ecosystem, ecosystem respiration (Re) is the most critical component determining large scale spatial and temporal variation in annual C balances (Valentini et al. 2000; Griffis et al. 2004; Flanagan and Johnson 2005; Lin et al. 2011). Therefore, to predict long-term trends in the C balance of an ecosystem, it is necessary to understand the responses of Re to management practices. Many studies have demonstrated that N fertilizer may decrease carbon dioxide (CO_2) emissions and promote C sequestration (Ladd et al. 1994; Smolander et al. 1994; Ma et al. 1999; Micks et al. 2004; Al-Kaisi et al. 2008), but in some cases N addition could increase or have no effect on CO_2 emissions (Entry et al. 1996; Conti et al. 1997; Willson et al. 2001; Kaye et al. 2005; Zhang et al. 2007). This inconsistency was attributed to: (1) Re is composed of plant autotrophic respiration and soil heterotrophic respiration, and they respond differently to N fertilizer; and (2) Re is controlled by soil temperature, soil moisture and plant physiological status (i.e. plant growth). For example, in some studies of semi-natural and natural vegetation, plant productivity did not respond to N addition, which suggests that these communities are phosphorus (P)-limited, or that changes in soil chemistry and species composition are slow, despite high N inputs (Morecroft et al. 1994; Bobbink 1998; Lee and Caporn 1998; Dupre et al. 2010).

Legume and non-legume intercropping systems can provide substantial amounts of N to plants and soil, which may reduce the need for industrial fertilizers (Ledgard and Steele 1992; Vance 1997; Drinkwater et al. 1998; Carlsson and Huss-Danell 2003; Gruber and Galloway 2008). The introduction of legumes at suitable seeding ratios helps to increase the productivity and quality of grass-based cropping systems (Hauggaard-Nielsen et al. 2001a; Chen et al. 2004; Ghaley et al. 2005; Lithourgidis et al. 2006). Caballero et al. (1995) found that mixture of common vetch (legume) and oat (grass) produced 34 % more dry matter than vetch monoculture, with no significant difference among mixture ratios. Ji (2008) also demonstrated that a 42:76 (kg hm^{-2}) seeding ratio of common vetch and oat produced 68.6 % and 15.7 % more dry matter than vetch and oat monocultures, respectively. The introduction of legume helps to improve soil fertility and biology (Vance 1997; Adgo and Schulze 2002; Carlsson and Huss-Danell 2003) by providing complementarities in the utilization of resources, such as light, water and nutrients (Jensen 1996; Hauggaard-Nielsen et al. 2001a, 2003; Mariotti et al. 2009). However, some reports indicate that grass-legume mixtures show higher respiration than grass-only pastures (Van Eekeren et al. 2009) due to a higher amount of active soil microbial biomass and higher N-mineralization in cereal-legume pastures compared to cereal-only pastures (Elgersma and Hassink 1997). Conversely, De Vries et al. (2006) found a higher microbial biomass in grass-only pastures than in grass-clover pastures under different seeding ratios.

To date, few studies have investigated the effect of N fertilizer on ecosystem respiration under mixed legume and grass pastures sown at different seeding ratios (Van Eekeren et al. 2009), and data are almost entirely lacking for alpine meadow regions of the Tibetan Plateau. Alpine meadows cover about 35 % of the Tibetan Plateau, which extends over 2.5 million km^2 and acts as a sink of CO_2, playing an important role in global C cycling (Zhao and Zhou 1999; Jian 2002; Cao et al. 2004; Li et al. 2007). With rapid economic and social development, the degradation of grasslands and the expansion of forage production have increased the pressure to cultivate alpine meadows for food and forage. In addition, considering the growing consumption demand of China's population, China's government has set a target for cutting emissions intensity (i.e. emissions of greenhouse gases (GHGs) per unit of Gross Domestic Product (GDP)) rather than a reduction in the absolute level of emissions. Thus, considering both the need to mitigate climate change and the need to meet consumption demand, there is a need to identify cost-effective ways to avoid increased GHG emissions (Burney et al. 2010). In view of this, understanding the Re intensity (i.e. Re per unit of aboveground biomass) of management options will contribute to identifying management strategies to balance forage production and environmental objectives in the alpine meadow areas of China. Understanding the effects of different seeding ratios of legume and grass on forage production and C balance in the alpine regions of the Tibetan plateau contributes to this objective.

Considering evidence that grass-legume mixtures may have higher Re than grass-only pastures (Van Eekeren et al. 2009) and that N fertilizer may increase C sources (Jassal et al. 2011), we hypothesized that although a combination of legume with grass and N fertilizer increases Re, the combination decreases Re intensity due to a greater increase in aboveground biomass compared to the increase in Re. The objectives of the present study were: (1) to examine the effects of different seeding ratios of common vetch and oat, N fertilizer and their interaction on Re during 2009 and 2010; (2) to investigate their effects on Re intensity; and (3) to determine the relationships between biotic or abiotic factors and Re in pastures in the alpine region.

Materials and methods

Study site

This study site is located at Haibei Alpine Meadow Ecosystem Research Station, Northwest Plateau Institute of Biology, Chinese Academy of Sciences (37°36′ N, 101°12′E, and 3,250 m above sea level). Mean annual precipitation is 580 mm with 80 % of precipitation concentrated in the growing season from May to September (Li et al. 2004). Mean annual air temperature is −1.7°C, and the lowest and highest monthly mean values are −15°C in January and 10°C in July. The soil is a clay loam, with an average depth of 65 cm which is classified as Mat-Gryic Cambisol (Chinese Soil Taxonomy Research Group 1995), corresponding to Gelic Cambisol (WRB 1998). Soil sampled in May 2007 had a total organic carbon (TOC) of 55.8 g kg^{-1}, total N content of 5.4 g kg^{-1}, total potassium (K) content of 13.0 g kg^{-1}, total P content of 0.7 g kg^{-1}, pH of 8.2 and bulk density in the layer 0–10 cm of soil of 1.05 g cm^{-3}. The native plant community is dominated by *Kobresia humilis, Elymus nutans, Stipa aliena, Gentiana straminea*, *Potentilla nivea* and *Festuca ovina*. Mean air temperature and total rainfall were 8.6°C and 308.8 mm in 2009, and 9.3°C and 410.8 mm in 2010 during the growing seasons from 15 May to 30 September. The seasonal rainfall distributions and temperatures are shown in Fig. 1.

Experimental design

An experimental site of 100 m×100 m was fenced in early May 2007. The mixed sown pastures and N addition experiments were initiated in 2009. Two main forages in the region, a legume (common vetch, *Vicia sativa L.*) and a grass (oat, *Avena sativa L.*), were selected for planting in the pasture ecosystem. The experimental plots (4.0 m×4.5 m) were laid out in a two × five factorial completely randomized design, i.e. two N fertilizer levels (fertilizer (F) and no-fertilizer (NF)) with five ratios of mixed forages. The seeding ratios of oat and common vetch were 0:150 (M1), 150:105 (M2), 300:75 (M3), 450:45 (M4) and 600:0 (M5) kg seeds ha^{-1}. To ensure the seeding mixes accurately reflected seeding establishment, the calculation of seed density was based on seeding germination rates. In total, ten treatments with three replicates were established by the end of May in 2009 and 2010. N fertilizer (69 kg N ha^{-1} as urea) was applied as additional fertilizer in the middle of July in 2009 and 2010. Each plot was separated by a 2 m-wide buffer zone.

Field sampling and measurement

Soil temperature and moisture

Soil temperature and soil moisture at 5 cm below the soil surface were monitored at each chamber when gas samples were collected. Soil temperature was measured using a digital temperature sensor (JM624 digital thermometer, Living–Jinming Ltd., China) and volumetric soil moisture was measured using a Time Domain Reflectometer (JS-TDR300, Meridian Measurement, USA).

Aboveground and belowground biomass

Aboveground biomass was measured by clipping a 1 m×1 m quadrat 20 cm away from the plot edge in each plot in late August of each year. Meanwhile, at the center of each quadrat two soil cores of 0–20 cm depth were collected using an 8 cm diameter soil auger to estimate belowground biomass. All soil samples were washed in the laboratory to remove the soil and belowground biomass was dried at 65°C for about 48 h to a constant weight.

Re measurement and Re intensity calculation

Ecosystem respiration was measured using a static closed opaque chamber and gas chromatography techniques (Lin et al. 2009). The static chamber consisted

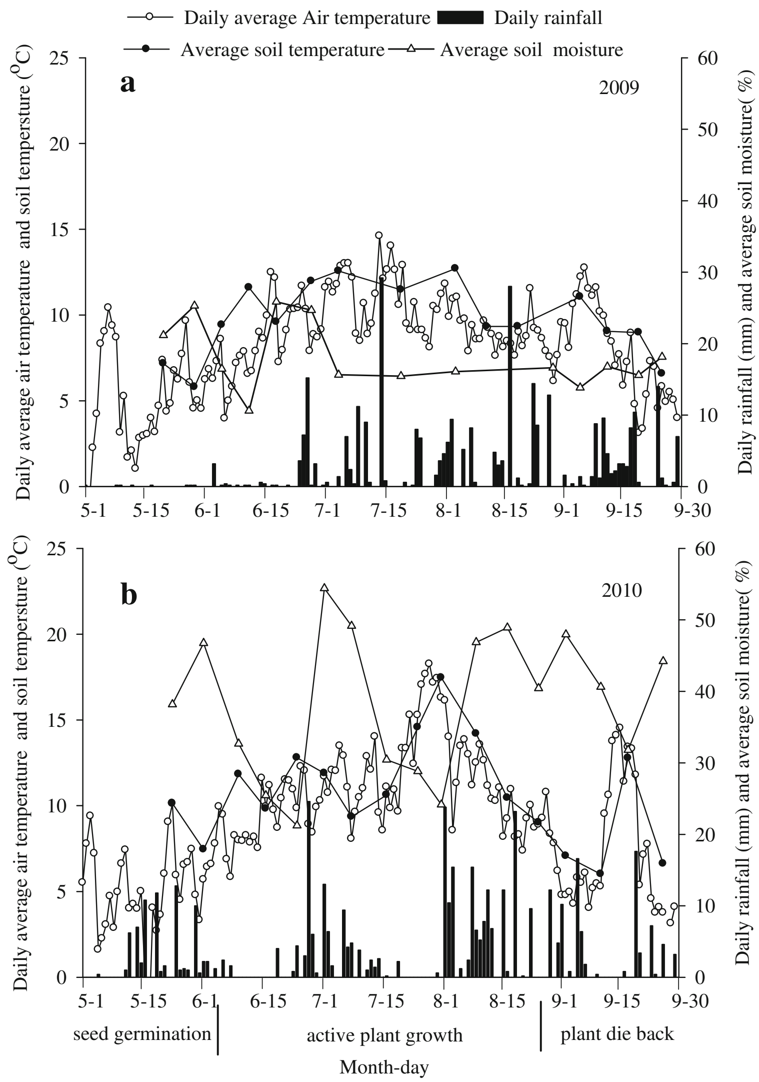

Fig. 1 Distribution of daily rainfall and daily average air temperature in 2009 (**a**) and 2010 (**b**). Average soil moisture and temperature at 5 cm soil depth for all treatments from May 1 to September 30 in 2009 (**a**) and 2010 (**b**)

of two parts, a stainless steel collar (0.4 m (length)× 0.4 m (width)×0.08 m (height)) with water groove to make the chamber airtight and a removable lid (0.4 m (length)×0.4 m (width)×0.4 m(height)), as described by Qi et al.(2007). The lid was made of stainless-steel, and a fan was installed inside of the top wall to ensure good air-mixing when the chamber was closed, and a silica gel pipe was connected to a syringe and a three-way stopcock for gas sampling. In May 2009, after sowing, one stainless steel collar was placed 20 cm away from the plot edge and inserted into the soil at 8 cm depth in each plot. There was a set of 10 collars and lids in one repeat. During flux measurements, the lid was sealed on the square collar. Re was measured every 7–10 days during the growing seasons in 2009 and 2010. Samples were collected from 09:00 a.m. to 11:00 a.m., representing 1-day average flux as described in previous reports (Lin et al. 2009; Jiang et al. 2010). Gas samples were collected with 100 ml plastic syringes every 10 min four times after the chamber was closed. All the gas samples were analyzed for CO_2 within 24 h using a gas chromatograph (HP Series 4890 plus, Hewlett Packard, USA). This device was equipped with a flame ionization detector (FID) for CO_2 analysis. The gas flux was calculated from the slope of the linear regression between concentration and time using the equation described by Song et al. (2003). Coefficients of determination (R^2) for all linear regressions were >0.98. The gas fluxes of the treatments and standard errors were calculated from three replicates for all observations. The monthly Re was estimated by calculating the average of 2 fluxes in May and the average of 4 fluxes in other months. The seasonal Re in 2009 and 2010 was estimated by calculating average fluxes over an experimental period with 18 flux observations in 2009 and 2010, respectively. Re intensity was estimated as the ratio of seasonal average Re to aboveground biomass in each year.

Statistical analysis

General Linear Model-Repeated Measures (SPSS 16.0, SPSS Inc., Chicago, IL, USA) was performed with seeding ratios and N fertilizer as the main factors (between-subject) and with sampling date as the within-subject factor including their interactions to test the effects of the main factors on soil temperature, soil moisture, daily and seasonal Re. Multi-comparison of least standard difference (LSD) was conducted for all measured variables within each sampling date using a two-way ANOVA with aboveground biomass or belowground biomass or seasonal average Re as the dependent variable and seeding ratios and N fertilizer as the main factors.

Simple correlation analyses and stepwise regression analysis were conducted to test the relationship between Re and biotic or abiotic factors with Re as the dependent variable, and soil temperature, soil moisture, aboveground biomass (AB), belowground biomass (BB) and BB/AB ratio as the independent variables. Results were judged to be significant when $p<0.05$.

Results

Soil temperature and soil moisture

During the observation periods, the seasonal dynamics of the average soil temperature of all treatments followed the pattern of daily air temperature as a single-peak curve (see Fig. 1). After a failure of rains for a long time, the average soil moisture of all treatments varied greatly and increased simultaneously with rainfall events (see Fig. 1). Daily average air temperature, daily rainfall, average soil temperature and soil moisture of all treatments were all significantly higher in all treatments in 2010 than in 2009 (see Figs. 1, 2a and b). Generally, the seeding ratio and N fertilizer did not significantly affect seasonal average soil temperature or soil moisture (see Table 1, Fig. 2a and b), and there was no significant effect of interactions between seeding ratio and N fertilizer on seasonal average soil temperature or soil moisture (see Table 1). However, the effects of seeding ratio on soil moisture varied with sampling date and year (see Table 1).

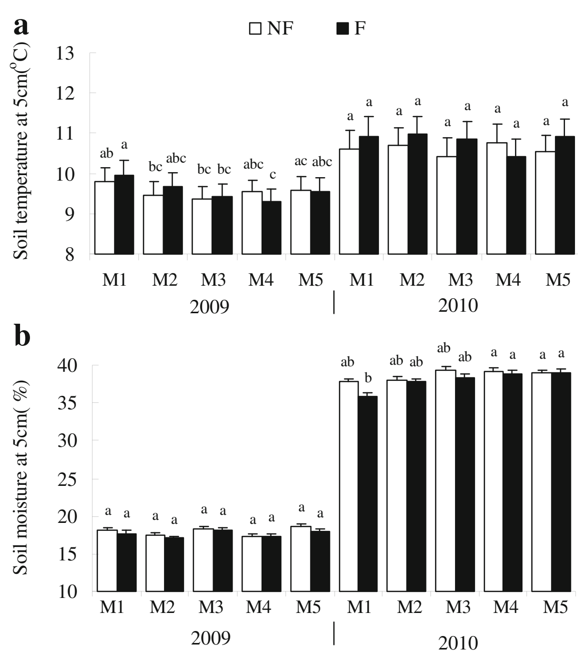

Fig. 2 Annual soil temperature (**a**) and soil moisture (**b**) at 5 cm soil depth under different treatments from May 2009 to September 2010. M1F, M1NF, M2F, M2NF, M3F, M3NF, M4F, M4NF, M5F and M5NF are the seeding ratios of oat and common vetch at 0:150, 150:105, 300:75, 450:45 and 600:0 with (F) or without N fertilizer (NF), respectively. Bars indicate mean ± 1SE. Different letters indicate significant difference at $p=0.05$ level

Table 1 Summary of repeated-measures ANOVA on the effects of year, day, mixture ratio and N fertilizer on soil temperature, soil moisture and ecosystem respiration (Re)

Model	Soil temperature		Soil moisture		Re	
	F	P	F	P	F	P
Year (Y)	70.817	<0.001	5214.000	<0.001	64.387	<0.001
Day (D)	306.852	<0.001	201.800	<0.001	242.471	<0.001
Mixture ratio (M)	0.484	0.747	1.112	0.378	4.341	0.011
Fertilizer (F)	0.241	0.629	1.267	0.274	1.169	0.292
Y*M	1.539	0.233	3.035	0.042	3.311	0.031
Y*F	0.320	0.579	1.188	0.289	0.069	0.796
D*M	0.771	0.878	1.374	0.064	4.198	0.001
D*F	0.356	0.985	0.556	0.876	1.169	0.291
M*F	0.569	0.688	0.016	0.999	0.378	0.822
Y*D	142.621	<0.001	372.111	<0.001	31.196	<0.001
Y*M*F	0.593	0.672	0.285	0.884	1.449	0.255
D*M*F	0.976	0.528	0.907	0.649	0.529	0.999
Y*D*M	0.618	0.790	1.878	0.001	1.529	0.010
Y*D*F	0.454	0.954	1.011	0.439	0.662	0.831
Y*D*M*F	0.863	0.741	1.276	0.122	0.438	1.000

Aboveground and belowground biomass

Seeding ratio ($p<0.001$), year ($p<0.001$) and their interaction ($p<0.001$) significantly affected aboveground biomass (see Fig. 3), but N fertilizer did not significantly affect it ($p=0.238$). Belowground biomass was not significantly affected by all treatments or their interactions over the 2 years (data not shown). In both 2009 and 2010, aboveground biomass was significantly lower for M1 compared with other treatments. The aboveground biomass of M3 and M4 was significantly higher than that of oat monocultures in 2009, both with and without N fertilizer (see Fig. 3).

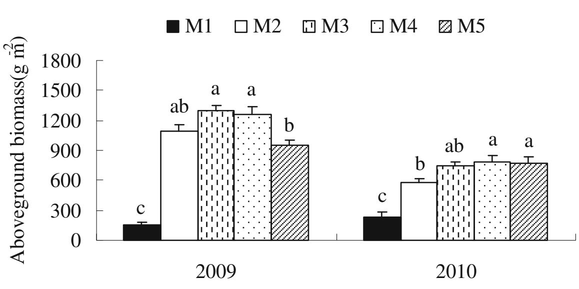

Fig. 3 Mean annual aboveground biomass in 2009 and 2010 under different seeding ratios, pooling data with (F) and without N fertilizer (NF). M1, M2, M3, M4, M5 are the seeding ratios of oat and common vetch at 0:150, 150:105, 300:75, 450:45 and 600:0, respectively. Bars indicate mean ± 1SE. Different letters indicate significant difference in every year at $p=0.05$ level

There was no significant difference in aboveground biomass between M4 and M3 in either year (see Fig. 3). Aboveground biomass was the highest for M3, which was 745.6 %, 18.6 %, 2.9 %, and 36.6 % higher than that of M1, M2, M4 and M5 in 2009. However, in 2010 it was highest for M4 which was 232.4 %, 37.5 %, 6.4 %, 2.7 % higher than that of M1, M2, M3 and M5. There was no significant difference between M3, M4 and M5 in the wetter year of 2010.

Ecosystem respiration (Re)

Seeding ratio and the interaction of seeding ratio and sampling date significantly affected Re, and their effects varied with year (see Table 1). N fertilizer did not significantly affect Re and there was no interaction between seeding ratio and N fertilizer during the growing seasons (see Table 1).

The highest value of daily Re occurred in August for all treatments except for the common vetch monoculture in 2009, which occurred in the middle of September (see Fig. 4a, b, c and d). Compared to the other seeding ratios, the common vetch monoculture had a significantly lower Re during the growing seasons until it reached its peak of daily Re (see Fig. 4a, b, c and d). N fertilizer significantly decreased the Re of M1 after fertilization in 2009 and from the end of June to the end of August in 2010 (see Fig. 4a, b, c and d).

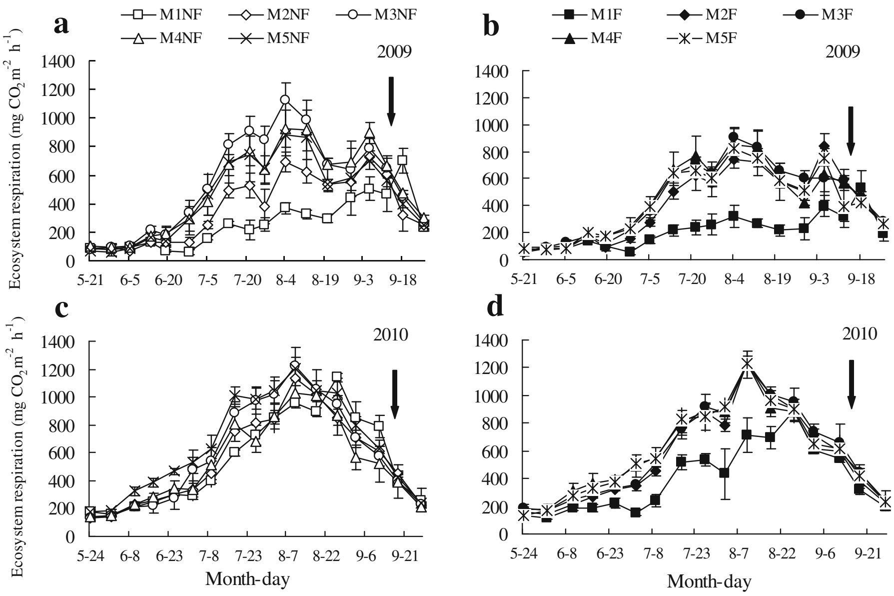

Fig. 4 Daily ecosystem respiration in different treatments in 2009 with (F) (**a**) and without N fertilizer (NF) (**b**) and 2010. with (F) (**c**) and without N fertilizer (NF) (**d**) M1F, M1NF, M2F, M2NF, M3F, M3NF, M4F, M4NF, M5F and M5NF are the seeding ratios of oat and common vetch at 0:150, 150:105, 300:75, 450:45 and 600:0 with (F) or without N fertilizer (NF), respectively. Bars indicate mean ± 1SE. ⟶ in the figures indicates the harvest date

The monthly average Re of M1 was significantly lower than other seeding ratios from July to September in 2009 (see Fig. 5a). There was no significant difference between the monthly average Re of M1 and M2 in June 2009, but they both had a significantly lower Re than other seeding ratios. From June 2009 to September 2009, there was no significant difference among the monthly average Re of M3, M4 and M5 (see Fig. 5a). The monthly average Re increased with an increase in the proportion of oat in the seeding mix in June 2010 (see Fig. 5b). M5 had the highest monthly average Re from June to September in 2010 compared with other seeding ratios, and M4 had a lower monthly average Re compared with M3 from July to September in 2010 (see Fig. 5b).

The seasonal average Re was 263.3, 214.5, 367.1, 404.6, 536.5, 446.9, 499.6, 443.7, 451.1 and 425.5 mg CO_2 m^{-2} h^{-1} during the growing seasons for M1NF, M1F, M2NF, M2F, M3NF, M3F, M4NF, M4F, M5NF and M5F in 2009, and the seasonal average Re was 544.4, 393.8, 545.8, 556.6, 589.7, 583.4, 526.3, 580.4, 654.4 and 585.1 mg CO_2 m^{-2} h^{-1} during the growing seasons for M1NF, M1F, M2NF, M2F, M3NF, M3F, M4NF, M4F, M5NF and M5F in 2010, respectively. N fertilizer only significantly decreased the seasonal average Re for M1 during the 2-year experimental period. Re was significantly lower for M1 than for the other treatments, Re was significantly lower for M2 than for M3 and M4, and there was no significant difference in Re among M3, M4 and M5 when data from with- and without- fertilizer treatments in 2009 were pooled (see Fig. 5c). Treatment M5 had a significantly higher seasonal average Re than M1, M2 and M4, and there was no significant difference for Re among M1, M2, M3 and M4 when data from with- and without- fertilizer treatments in 2010 were pooled (see Fig. 5c). Seasonal average Re was significantly higher in all treatments in 2010 than in 2009.

Ecosystem respiration (Re) intensity

Seeding ratio (p=0.001) and year (p=0.038) significantly affected Re intensity, but there was no interaction between seeding ratio and year (p=0.594). N fertilizer did not significantly affect Re intensity (p=0.223) and there was no interaction between seeding ratio and N fertilizer (p=0.201) during the growing seasons. However, M1 had significantly higher Re intensity compared with other treatments due to a greater decrease in aboveground biomass compared to the decrease in Re (see Fig. 6b). There was no significant difference among the Re intensity of M2, M3, M4 and M5 regardless of N fertilizer (see Fig. 6b). However, N fertilizer significantly decreased Re intensity for M1 because it significantly decreased seasonal average Re and increased aboveground biomass (see Fig. 6a and b). Re intensities in 2010 were significantly higher than in 2009 for all treatments due to a greater decrease in aboveground biomass and the

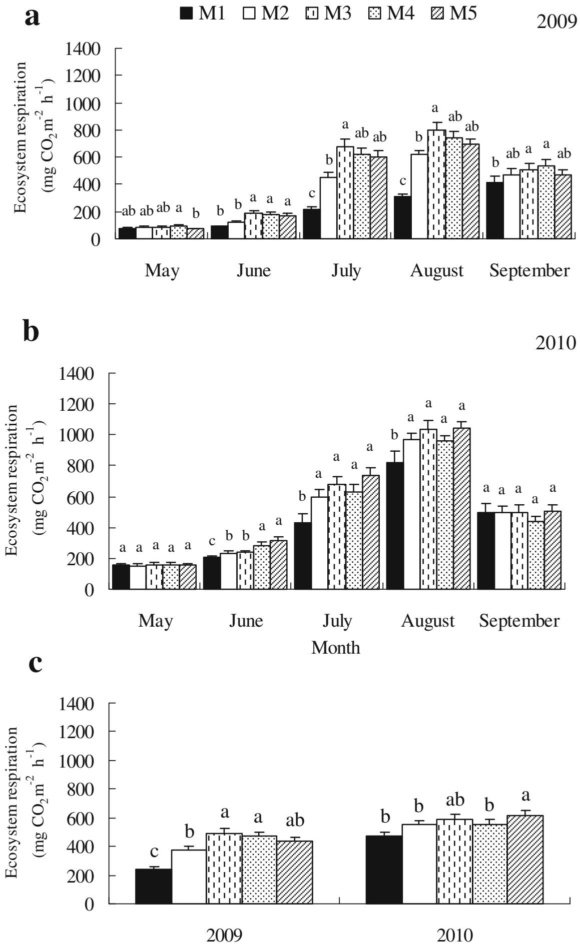

Fig. 5 Monthly (**a** and **b**) and annual (**c**) average ecosystem respiration of different seeding ratios, pooling data with (F) and without N fertilizer (NF). M1, M2, M3, M4, M5 are the seeding ratios of oat and common vetch at 0:150, 150:105, 300:75, 450:45 and 600:0, respectively. Bars indicate mean ± 1SE. Different letters indicate significant difference at p=0.05 level

increase in Re due to abundant rainfall in 2010 and its inappropriate distribution (see Figs. 6a, b and 1).

Relationship between environmental factors and Re

Although the correlations between daily Re and soil temperature and soil moisture were significant for all treatments, except M1F, soil temperature and soil moisture only explained about 15 % of the variation in daily Re when data was pooled from both years (see Table 2). Moreover, their influence on daily Re varied with different treatments (explaining 8–25 % of the variation in daily Re), with the smallest influence on M3NF and M4NF (see Table 2). Generally, N fertilizer increased the dependence of daily Re on soil temperature and soil moisture compared with no fertilizer, although the strength of this relationship varied with different seeding ratios.

Soil moisture, and/or aboveground biomass and/or soil temperature explained 45–93 % of the variation in seasonal average Re, with variation between treatments (see Table 3). Although soil moisture and aboveground biomass were the main controlling factors on seasonal average Re when all data for all treatments were pooled, soil temperature (for M2F and M5F) and belowground biomass (for M3F, M4F and M4NF) and the ratio of BB/AB (for M2NF and M2F) had significant influences on seasonal average Re. For example, soil temperature explained 93 % of the variation in seasonal average Re for M5F (see Table 3). However, no significant relationships between abiotic and biotic factors and seasonal average Re were found for M1NF, M3NF or M5NF. Based on the indexes of the regression, we found that N addition increased the dependence of seasonal average Re on soil temperature and altered the direction of the effect of aboveground biomass on seasonal average Re (see Table 3).

Discussion

Effect of mixed pasture on Re

Mixed pastures of common vetch with oat increased aboveground biomass and seasonal average Re regardless of the seeding ratios between legume and grass or N fertilizer when compared with monocultures of common vetch (see Figs. 3 and 5c). This only partially supported our hypothesis, because there was a lack of N fertilizer effects. Compared with common vetch monoculture, the positive effect on Re of the grass-legume mixtures may be attributed to two causes. On the one hand, for grass-legume mixtures, legumes can supply nutrients to grass through the soil food web (Van Eekeren et al. 2009) to stimulate plant growth, thus increasing plant respiration. On the other hand, the increasing percentage of oat in mixed pastures stimulated the symbiotically fixed N_2 in the common vetch (Nyfeler et al. 2011), leading to a higher amount of active soil microbial biomass and higher N-mineralization in mixtures than in monoculture pasture, thus increasing soil respiration (Elgersma and

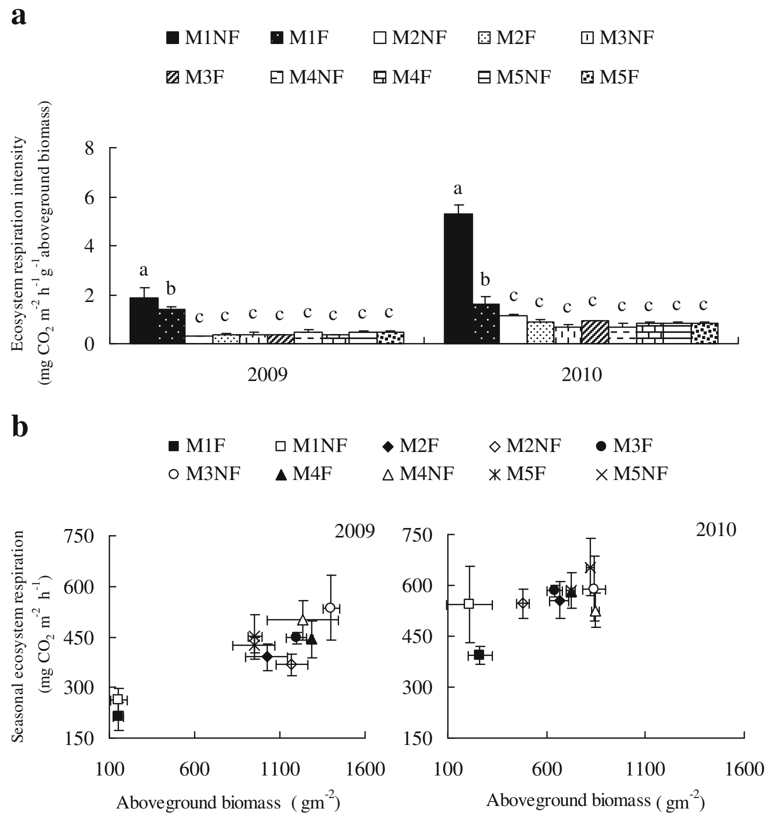

Fig. 6 Seasonal ecosystem respiration intensity of different treatments in 2009 and 2010 (**a**) and the relationship between seasonal ecosystem respiration and aboveground biomass (**b**). M1F, M1NF, M2F, M2NF, M3F, M3NF, M4F, M4NF, M5F and M5NF are the seeding ratios of oat and common vetch at 0:150, 150:105, 300:75, 450:45 and 600:0 with (F) or without N fertilizer (NF), respectively. Bars indicate mean ± 1SE. Different letters indicate significant difference at p=0.05 level

Hassink 1997). Since Re depends on plant respiration and soil respiration, the two components moving in the same direction resulted in higher Re in mixed plots than in common vetch monoculture plots. Although there were almost no significant differences in aboveground biomass (see Fig. 3) and seasonal average Re (see Fig. 5c) for M3 (i.e. 300:75 ratio of oat: common vetch) and M4 (i.e. 450:45 ratio of oat: common vetch) treatments regardless of N fertilizer, the M3 treatment provided greater legume production compared with M4 treatments (data not shown). Furthermore, it is known that legumes have a higher concentration of crude protein than grass (Lithourgidis et al. 2006). In addition, compared to oat monoculture,

Table 2 Models of daily ecosystem respiration (Re), soil temperature (ST) and soil moisture (SM) through stepwise regression under different seeding ratios with or without N fertilizer

Treatment	Fertilized			Unfertilized		
	Linear model	R^2	P	Linear model	R^2	P
Pooled data$^{(1)}$	Re=−105.281+36.299ST+6.301SM	0.17	<0.001	Re=−91.532+5.377ST+1.162SM	0.14	<0.001
M1$^{(2)}$	Re=195.119+4.171SM	0.06	0.068	Re=−164.884+29.175ST+9.859SM	0.17	<0.001
M2$^{(3)}$	Re=−58.547+6.696ST+24.682SM	0.13	<0.001	Re=−30.864+6.3955ST+22.549SM	0.12	<0.001
M3$^{(4)}$	Re=−99.190+43.817ST+4.797SM	0.19	<0.001	Re=138.862+40.04ST	0.09	0.004
M4$^{(5)}$	Re=−190.186+50.749ST+6.140SM	0.25	<0.001	Re=188.22+29.987ST	0.08	0.007
M5$^{(6)}$	Re=−164.301+44.958ST+6.459SM	0.22	<0.001	Re=−211.854+53.273ST+7.133SM	0.21	<0.001

(1): all seeding ratios pooled. (2), (3), (4), (5), (6) are the seeding ratios of oat and common vetch at 0:150, 150:105, 300:75, 450:45 and 600:0, respectively

Table 3 Models of seasonal average ecosystem respiration (Re) during the growing seasons, aboveground biomass (AB), belowground biomass (BB), the ratio of BB and AB (BB/AB), soil temperature (ST) and soil moisture at 5 cm (SM) through stepwise regression under different seeding ratios with and without N fertilizer

Treatment	Fertilized			Unfertilized		
	Linear model	R^2	P	Linear model	R^2	P
Pooled data[(1)]	Re=−103.577+14.408SM-75.549AB+53.411ST	0.79	<0.001	Re=89.666+9.802SM+0.161AB	0.45	<0.001
M1[(3)]	Re=33.245+10.136SM	0.81	0.014	None[(2)]		
M2[(4)]	Re=−1090.36+167.492ST-175.937BB/AB	0.96	0.019	Re=278.067+217.295BB/AB	0.92	0.002
M3[(5)]	Re=432.962+7.083SM-0.165BB	0.97	0.008	None		
M4[(6)]	Re=863.596-0.495BB	0.7	0.039	Re=864.918-0.473BB	0.87	0.007
M5[(7)]	Re=−762.438+123.848ST	0.93	0.002	None		

(1): all seeding ratios pooled. (2): have no relationship. (3), (4), (5), (6), (7) are the seeding ratios of oat and common vetch at 0:150, 150:105, 300:75, 450:45 and 600:0, respectively

aboveground biomass was significantly greater for mixed pastures in the drought year 2009, but there was no significant difference between mixed pastures and oat monoculture in the wetter year of 2010, indicating that the mixture of common vetch and oat has advantages over oat monoculture, especially in drought years These results suggest that planting mixed pastures of oats and legume is a promising strategy to address the severe shortage of protein forages in the alpine region.

Meanwhile, we also observed that the effect of seeding ratios on Re varied with year (see Table 1). Seasonal average Re was higher in 2010 than in 2009, which is consistent with other studies (Xia et al. 2009; Lin et al. 2011). Stepwise regression analysis showed that Re was mainly controlled by soil moisture and/or soil temperature, plant aboveground and belowground biomass (see Table 3), which is consistent with other studies (Verchot et al. 2000; Flanagan and Johnson 2005; Suh et al. 2009; Schrier-Uijl et al. 2010). In 2010 plant respiration in all treatments except common vetch monoculture could be lower than plant respiration in 2009 due to the lower aboveground biomass (see Fig. 3). We suggest that this phenomenon is related to the following factors: (1) there was a heavy rainfall of 32.2 mm during the period of seed germination in June 2010 (i.e. from 24 May to 4 June 2010, Fig. 1), which caused air temperature to fall to below 3.5°C and which adversely affected the growth of seedlings; and (2) there were two long dry periods (i.e. from June 9 to June 23 and from July 17 to July 31 in 2010) in the otherwise wetter year of 2010 (see Fig. 1), which occurred during the periods of more active plant growth. During these events, extreme air temperatures and soil temperature above the optimal growth temperature occurred. It is likely that the main factor controlling aboveground biomass is not season-averaged soil moisture or rainfall values, but soil temperature and the distribution of water availability. This is in line with results of our former warming experiment which showed a negative relationship between soil moisture and aboveground biomass in the region (unpublished data). Due to the higher soil temperature and moisture in 2010 compared to 2009, soil respiration in 2010 was higher than in 2009, as was also shown in Lin et al. (2011). Usually, heterotrophic respiration is more variable than autotrophic respiration. The increase in soil respiration could offset the decrease in plant respiration in 2010 compared to 2009, and thus lead to a higher Re in 2010 compared to 2009. As the plant growth rate of common vetch monoculture was lower than other treatments, the effect of the distribution of water availability in 2010 was minimal. The higher rainfall and temperature increased both plant and soil respiration in 2010 compared to 2009. The contribution of both these two components of Re need to be further investigated.

Effect of N fertilizer on Re

Some studies have reported that N fertilizer tends to inhibit soil CO_2 emission, stimulate plant growth and thus promote ecosystem C sequestration (Micks et al. 2004; Magnani et al. 2007; Mo et al. 2008) However, this study found that N fertilizer had no significant effect on aboveground biomass and Re. Some other studies of semi-natural and natural vegetation also reported that plant productivity did not respond to N addition, suggesting that these communities are P-limited (Morecroft et al. 1994; Bobbink 1998; Lee and Caporn 1998). In another P fertilizer experiment (unpublished data), we also found that P fertilizer stimulated plant growth. The lack of N addition effects on Re could be attributed to counteracting plant and soil respiration response patterns. Increased N uptake following N fertilizer would influence metabolic processes in living cells, which is expected to induce short-term increases in plant respiration (Ryan 1991; Vose et al. 1997; Jassal et al. 2011) while N fertilizer decreases soil respiration via decreased microbial biomass and activity (Vose et al. 1997; Frey et al. 2004; DeForest et al. 2004; Mo et al. 2008). Although N fertilizer did not significantly affect plant production or seasonal average Re for all treatments, N fertilizer significantly decreased the Re of common vetch monoculture during the middle of August–September in 2009 and July–August in 2010. This may be because N fertilizer suppressed the N fixation of common vetch monoculture during these periods (Carlsson and Huss-Danell 2003), which may reduce rhizo-respiration. Therefore, in the future N fertilizer use in forage production could be reduced through the establishment of mixed pastures using grass and legumes in the alpine region.

Re intensity

In our study, compared with the common vetch monoculture, we found that mixed pastures significantly decreased Re intensity regardless of N fertilizer. This finding is mainly due to the greater increase in aboveground biomass compared to the increase in Re in mixed pastures compared to common vetch monoculture (see Fig. 6a and b), which partially supported our hypothesis. Although mixed pastures did not significantly reduce Re intensity compared with oat monoculture pasture, the mixed pastures have the advantage over oat monoculture pasture that they can increase forage quality as well as quantity, especially in drought years. N fertilizer significantly decreased the Re intensity of common vetch monoculture especially in the wetter year (2010), but had no significant effect on the Re intensity of the mixed pastures. To mitigate the future contribution of agriculture to climate change, approaches for increasing forage yields and reducing the need for industrial fertilizers are paramount (Tilman et al. 2002; Robertson and Vitousek 2009; Vitousek et al. 2009; Burney et al. 2010). The contradiction between the need for increased forage production and environmental problems induced by N fertilizer can be resolved by mixing N-fixing and non-N-fixing crop species (Loiseau et al. 2001; Hauggaard-Nielsen et al. 2003). On the one hand, this can reduce grazing pressure and increase carbon storage in natural alpine meadows, since aboveground biomass is about 5 times higher for mixed pastures than for the natural alpine meadows in the region. On the other hand, it can reduce Re intensity and thus balance the dual needs for economic development and ecological services (Hauggaard-Nielsen et al. 2001b; Temperton et al. 2007).

Conclusion

This study was the first to test the hypothesis that although mixtures of legume and grass increase ecosystem respiration (Re), they decrease Re intensity due to greater increases in aboveground biomass compared to increases in Re. We found that the mixed pastures of common vetch and oat reduced Re intensity compared with common vetch monoculture. Although there were no significant differences in Re intensity between the mixed pastures and oat monoculture, the mixed pasture provided higher forage production with higher quality forages (i.e. higher crude protein content from legumes) in drought year 2009. N fertilizer did not significantly affect plant production and Re. Therefore, our results suggest that planting mixed pastures of legume and grass without N fertilizer is a preferable way to limit the GHG intensity of forage supply in the alpine region.

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