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EFFECTS OF WARMING AND NITROGEN ADDITION ON SOIL RESPIRATION, MICROBIAL PROPERTIES AND PLANT GROWTH IN GRASSLAND ECOSYSTEMS

Abstracts

Climate warming and atmospheric nitrogen deposition are two important components of global changes, which have profound impacts on the carbon cycle of terrestrial ecosystems. In the context of global changes, microbes and plants produce carbon feedback to the atmosphere through respiration. Therefore, a comprehensive understanding of the responses of soil respiration, microbial community and plant growth to climate change is of great significance for the parameterization of earth process models and the accurate prediction of the future global carbon cycle. Grassland, one important biota in terrestrial ecosystems, accounts for 40% of the terrestrial vegetation cover. However, the study of key soil carbon processes in grassland ecosystems in areas sensitive to climate change (high latitude or high altitude) is still lacking. Thus, we conducted field manipulative warming and nitrogen addition experiments in a semi-arid alfalfa-pasture and a fenced semi-arid natural grassland in the Loess Plateau of China, and evaluated the responses of above- and belowground biomass distribution and production to long-term (10 years) geothermal warming in a sub-arctic grassland. Open-top chambers were used to elevate temperature passively and geothermal warming was achieved due to an earthquake including three soil temperature treatments (+0 ºC, +3.6 ºC and +7.6 ºC) above soil ambient. N was enriched at a rate of 4.42 g m^{-2} yr^{-1} with NH_4NO_3. The main findings are as following:

(1) During April 2014 to March 2016 in the semi-arid alfalfa-pasture, N addition increased Rs by 14% over the two-year period; and warming stimulated Rs by 15% in the non-growing season, and inhibited it by 5% in the growing season, which can be explained by decreased plant coverage and soil water. The main effect of N addition did not change with time, but that of warming changed with time, with the stronger inhibition observed in the dry year. When N addition and warming were combined, an antagonistic effect was observed in the growing season, whereas a synergism was observed in the non-growing season. Overall, warming and N addition did not affect the Q_{10} value over the two-year period, but these treatments significantly increased the

Q10 value in the growing season compared with the control treatment. In comparison, combined warming and nitrogen addition significantly reduced the Q_{10} value compared with the single treatment. These results suggest that the negative indirect effect of warming-induced water stress overrides the positive direct effect of warming on Rs. Our results also imply the necessity of considering the different Rs responses in the growing and non-growing seasons to climate change to accurately evaluate the carbon cycle in the arid and semi-arid regions.

(2) During April 2015 and December 2016 in the fenced natural grassland, Warming significantly decreased Rt and Rh by 7.4% and 9.5%, respectively, but had no significant effect on Ra. N addition significantly stimulated Ra by 34%, whereas it inhibited Rh by 11% and had no significant effect on Rt. N addition significantly increased the contribution of Ra to Rt by 10%. Warming decreased the Q_{10} values of Rt and Rh but had no significant effect on Ra. N addition significantly increased the Q_{10} values of Rt and Rh, whereas it decreased the Q_{10} values of Ra. The combination of warming and N addition had a synergistic effect on the cumulant of Rh, whereas it had an antagonistic effect on Ra. No interactive effect between warming and N addition was observed on Rt. Our results emphasized that the Ra and Rh responded differently to warming and N addition. Our findings suggested that Rt has the potential to resist climate warming and increasing N deposition by differentiating the responses of its inherent components.

(3) During April 2015 and December 2017 in the fenced natural grassland, Warming did not affect microbial biomass or the composition of microbial functional groups. However, warming significantly decreased microbial respiration, directly resulting from soil pH decrease driven by the co-mediation of aboveground biomass increase, inorganic nitrogen increase and moisture decrease. These findings highlight that the soil microbial community structure of semi-arid grasslands resisted the short-term warming by 2 ^{o}C, although its metabolic rate declined.

(4) In the sub-arctic grassland, ten years soil warming did not increase standing root biomass and even fine root production, as well as decreased aboveground biomass and production. Structure equation modeling (SEM), used to understand underlying mechanisms, suggest that the reduction in fine root production was mainly attributed to the decreased aboveground biomass and the reduced soil organic carbon (magnitude index of soil organic matter) and total nitrogen. Long-term soil warming did not significantly change root-shoot ratio attributed to the balance between favorable

thermal and unfavorable substrate C and N environments for plant growth. These counterintuitive responses suggest an urgent need to improve our understanding of the comprehensive mechanisms underlying the responses of subarctic grasslands to long-term soil warming.

These results could provide important data support and theoretical basis for the parameterization of earth process models and more accurate prediction of future global carbon cycle.

Keywords: climate warming, nitrogen addition, soil respiration, soil microbial community, plant growth

EFFECTEN VAN OPWARMING EN STOKSTOFADDITIE OP BODEMRESPIRATIE, MICROBEN EN PLANTENGROEI IN GRASLANDECOSYSTEMEN

Samenvatting

Klimaatopwarming en atmosferische stikstofdepositie zijn twee belangrijke Global Change factoren die een grote impact uitoefenen op de koolstofcyclus van terrestrische ecosystemen. Door meer of minder koolstof op te nemen of terug af te geven aan de atmosfeer, bepalen planten en microben de sterkte en teken van de feedback tussen klimaatopwarming en de koolstof cyclus. Daarom is het belangrijk om de responsen van bodemrespiratie, microbiële gemeenschappen en plantengroei op klimaatverandering en stikstofdepositie ten gronde te begrijpen. Deze kennis zal dan helpen om globale klimaatmodellen te veranderen of beter te parameteriseren, waardoor de voorspellingen van het toekomstig klimaat accurater worden. In deze thesis werd onderzoek gedaan in opwarmingsexperimenten in drie verschillende graslandecosystemen. In twee ecosystemen werd opwarming gesimuleerd met behulp van open-top kamers en in een derde werd gebruik gemaakt van een natuurlijke bodemtemperatuursgradiënt om de veranderingen in ecosysteemprocessen bij huidige en toekomstige temperatuur te bestuderen. De effecten van stikstofadditie werden bestudeerd door een grasland aan te rijken met 4.42 g m^{-2} yr^{-1} NH_4NO_3. De belangrijkste resultaten waren:

1) In een grasland ingezaaid met luzerne zorgden hogere temperaturen en stikstofadditie voor grotere seizoenale schommelingen in bodemvocht, waardoor bodemrespiratie ook meer fluctueerde. Dit toont aan dat bodemrespiratie doorheen het hee jaar moet gemeten worden om de respons op temperatuur en stikstof juist in te schatten.

2) Terwijl we een synergistisch interactie-effect vonden van opwarming en stikstofadditie op heterotrofe bodemrespiratie in het onbeheerde, semi-ariede grasland, was er een antagonistich interactie-effect op de autotrofe bodemrespiratie, met als resultaat dat de volledige bodemrespiratie geen interactie-effect vertoonde. Met andere woorden, de bodemrespiratie in dit grasland verandert niet indien de klimaatopwarming gepaard gaat met verhoogde stikstofdepositie, maar de koolstofstocks zullen

waarschijnlijk wel veranderen door het synergistische effect op de microben.

3) Opwarming had geen effect op de microbiële biomassa of gemeenschaps-samenstelling, al daalde de respiratie wel doordat de pH daalde door de gezamenlijke effecten van meer actieve vegetatie en veranderingen in inorganische stikstof en watergehalte. Dit suggereert dat adaptatie van de microbiële gemeenschap belangrijk was in dit ecosysteem.

4) Ondanks de versnelde decompositie en mineralizatie in het lange-termijn opwarmingsexperiment in een subarctisch grasland zorgde dit niet voor hogere productiviteit; in tegendeel, de bovengrondse- en wortelproductie verlaagde. Er was geen effect op de wortel-scheutverhouding. Deze counter-intuïtieve observaties duiden op een onvolledige theoretische basis voor het parameterizeren van de vegetatiemodules van globale klimaatmodellen.

Keywords: klimaatopwarming, stikstofadditie, bodemrespiratie, microbiële gemeenschappen, plantengroei

Contents

Chapter 1 Introduction

Global change is a concept developed by the international union for science to describe global environmental changes caused by natural and human factors, including climate change, changes in the oceans and water resources, changes in the land, changes in the composition of the atmosphere and changes in ecosystems. Climate warming and atmospheric nitrogen deposition are two important components of current global changes.

1.1 Background

1.1.1 Climate warming

In the 19th century, the question of whether humans were experiencing global warming or the beginning of the ice age was first studied and discussed as a scientific question, but there was widespread controversy. By the beginning of the 20th century, the controversy had gained widespread attention. In the 1980s, the debate spilled over into the media, the public, and politics. The debate had become emotional at the time as both proponents and opponents of global warming held their ground (Khandekar et al., 2005).

As a noted climatologist, Roger Revelle in 1957 wrote: “humans are currently carrying out a largescale geophysical experiment that is not possible in the past nor be reproduced in the future. Within a few centuries, we are releasing high concentrations of organic carbon stored in sedimentary rocks for hundreds of millions of years into the atmosphere and oceans.... ”(Revelle and Suess, 1957). To answer the global warming debate more comprehensively, the United Nations Environment Program (UNEP) joined with the World Meteorological Organization (WMO) in 1988 to form the Intergovernmental Panel on Climate Change (IPCC). The IPCC's motivation was to provide authoritative scientific assessments that did not reflect the scientific consensus unduly influenced by extreme positions. The scientific framework of IPCC was set out in the 1996 and its climate change reports in 2001 led to the Kyoto Protocol, which aims to reduce emissions of greenhouse gases (especially carbon dioxide) from burning fossil fuels. Finally, the IPCC made a very strong statement in 2006 that we were 95%

confident that the most recent (late last century) warming was mainly caused by anthropogenic emissions (such as the burning of fossil fuels). In 2016, 178 countries signed the Paris Agreement on Climate Change, promising to limit global warming to no more than 2 degrees Celsius. Nowadays, many student groups organize climate marches, and public awareness of climate change has been widely raised, effectively urging policymakers to adopt more aggressive policies to mitigate climate change (Thackeray et al., 2020).

According to the 5th assessment report of IPCC, the global average temperature increased by 0.65 ^{o}C to 1.06 ^{o}C during the period of 1880-2012, and the increased magnitude of the average temperature during the two periods of 1850-1900 and 2003-2012 was 0.72 ^{o}C to 0.85 ^{o}C. Compared with the period of 1850-1900, the global surface temperature would increase by 1.4-4.5 ^{o}C by the end of the 21st century (IPCC, 2013). The warming trend is inconsistent across the globe, and the warming effect is more pronounced in the regions with high latitudes or altitudes (Thomas et al., 2004). The latest IPCC assessment shows that the global average temperature has now risen by 1 ^{o}C and stresses that tougher measures should be taken to limit the increase to 1.5 ^{o}C by the end of this century (Allen et al., 2018). The report also suggests that limiting the warming to 1.5 ^{o}C would reduce sea level rise by 10cm compared with the previous recommendation of 2 ^{o}C, beneficially protect ecosystem biodiversity, and effectively preventing more species extinctions.

1.1.2 Nitrogen deposition

The reactive nitrogen from the atmosphere deposited as NHx and NOx to ecosystems is called nitrogen deposition. Atmospheric nitrogen deposition was divided into wet (rain and snowfall) and dry atmospheric nitrogen deposition determined by its deposition means. Nitrogen composition in wet atmospheric nitrogen deposition back to the ground by precipitation are mainly NO_3^- and NH_4^+ and a small amount of soluble organic nitrogen. Nitrogen composition in dry atmospheric nitrogen deposition back to the ground by dust are gaseous NOx, solid ammonium nitrogen and nitrogen adsorbed on the surface of other solid particles.

Since 1970, the population in the world has increased by 78%, while the amount of reactive nitrogen produced has increased by 120%. As Delwiche says, trying to keep the nitrogen cycle properly balanced while feeding a growing world population will require human ingenuity. Thirty-five years later, Dobermann and Cassman pointed out that failure to prevent the loss of cereal crops and increase nitrogen efficiency in one of the world's most important agricultural systems could cause serious damage to environmental services on a local or regional even global scale, as a result of significant increases in the reactive nitrogen load in the environment.

Nitrogen deposition has attracted more and more attention due to its adverse effects on ecosystems such as soil acidification and imbalance of nitrogen and phosphorus in ecosystems (Basto et al., 2015; McPhee et al., 2015). It is estimated that the global emission of active nitrogen was only 15 Tg yr^{-1} in 1860, which increased to 156 Tg yr^{-1} in 1995 and 187 Tg yr^{-1} in 2005 (Galloway et al., 2008). In the US and Europe, nitrogen deposition has stabilized since the late 1980s or early 1990s due to strict legal constraints (Goulding et al., 1998; NADP, 2000). In Asia, the reactive nitrogen emission increased from 14Tg yr^{-1} in 1961 to 68 Tg yr^{-1} in 2000 and is expected to reach 105 Tg yr^{-1} in 2030 (Zheng et al., 2002). Galloway and Reay et al. have shown that East Asia will be the region with the largest nitrogen deposition in the world by the end of the 21st century, with China accounting for a large proportion (Galloway et al., 2004; Reay et al. 2008). Since the 1980s, China's anthropogenic nitrogen emissions have significantly increased, seriously affecting local air quality and causing increase of nitrogen deposition (Zhang et al., 2007; Zhang et al., 2009). The emissions of NOx and NH_3 in China increased from 8.4 Tg yr^{-1} and 10.8 Tg yr^{-1} in 1990 to 11.3 Tg yr^{-1} and 13.6 Tg yr^{-1} in 2000, respectively (Wang et al., 1996; Sun et al., 1997; Streets et al., 2003). The total nitrogen deposition was 12.64 kg N ha^{-1} yr^{-1} in the 1960s, and reached 20.07 kg N ha^{-1} yr^{-1} in the first decade of the 21st century (Lu and Tian, 2014).

1.2 Effects of warming on grassland ecosystems

1.2.1 Effects of warming on plant growth

Plants promote their own growth through photosynthesis. , Plant phenology is changing with global warming, inducing the growth of different vegetation changes accordingly. A large number of field manipulative experiments have shown that warming can both promote and inhibit plant growth. De Boeck et al. (2008) found that the warming significantly reduced the biomass of grassland ecosystem after three years of warming experiments. Zhou et al. (2000) showed that the increased temperature reduced the biomass of caraway meadow weeds by 21.13%. However, Whittington et al. (2013) found that the aboveground biomass of two leguminous plants was significantly increased by 15-year warming on a grassland in the north of the United

States. Lemmens et al. (2008) found warming significantly increased the flowers biomass due to earlier onset of growing season in an artificial grassland. A 5-year warming and drought experiments in two grassland ecosystems with different soil conditions suggested that warming alone significantly increased above-ground biomass in the second three years (Grime et al., 2000). Besides, the response of plant growth to temperature may be delayed. Sherry et al. (2018) demonstrated that the total plant biomass increased significantly under warming, but plant growth delayed the temperature rise in a tallgrass prairie. Ye et al. (2014) found that the net primary productivity (NPP) of grassland ecosystems was significantly increased under the single warming scenario through a climate change simulation study based on four plantation type ecosystems in the Qinghai-Tibet Plateau. Zhou et al. (2000) investigated the effects of simulated warming on vegetation in an alpine meadow ecosystem by the International Tundra Program (ITEX) and found that warming significantly increased the height and coverage of dominant species.

1.2.2 Effects of warming on plant and microbial communities

Temperature is an important factor that determines the community succession, in which the change of community structure is the most obvious reflection and symbol in the succession process. Global warming causes plant phenology to advance or delay due to rising surface temperatures, ultimately inducing the change of community structure in ecosystems (Pugnaire et al., 2019). Lemmens et al. (2008) found that warming increased species richness by 39.4 %. Zhou et al. (2000) also found that the community structure changed due to the warming, which increased the density of most species, but decreased the density of positive plants and shrubs in a Kobresia humilis meadow. Hou et al. (2013) conducted a 2-year simulated warming and rainfall experimental in a desert grassland in North China where annual plants were the dominant species, and found that warming significantly reduced the species richness and increased the C4 herbs density such as Cleistogenes squarrosa. Warming has not only an effect on plant community structure, but also on microbial community structure. Luo et al. (2014) conducted a 10-year warming experiment in a temperate grassland in the Midwest of the United States. Through genetic analysis, they found that the soil microbial community structure affected by warming was significantly changed. Zhang et al. (2014a) conducted a 3-year warming experiment in three alpine grasslands in Qinghai region of The Qinghai-Tibet Plateau. Through phospholipid fatty acid map analysis (PLFA), they found that the soil microbial community structure in the 0-10cm soil depth significantly changed under warming in the alpine marsh meadow and the alpine meadow, and the ratio of fungi to bacteria significantly decreased. Wang et al. (2012) conducted a warming experiment in an alpine meadow on the Qinghai-Tibet Plateau in Sichuan, and found that 1.17 ^{o}C soil warming increased the relative soil bacterial content by 8.8% but decreased the fungal content by 17.48%, as well increased the ratio of bacteria and fungi from 7.3 to 9.6 and the total soil microbial PLFAs by 34.58%.

1.2.3 Effects of warming on soil chemical properties

Soil chemical properties is an important constraint to ecosystem succession and response to environmental change. As a result of climate warming, the concentration of CO_2 in the atmosphere increases, which further affects the soil nitrogen mineralization, the decomposition of organic matter, and changes the soil nutrients availability and migration, thus changing the ecosystem structure and function. A 4-year warming experiment in a semi-arid grassland in the center of the Iberian Peninsula showed that warming increased soil nitrogen availability and the ratio of inorganic nitrogen to organic nitrogen, and indirectly affected the nitrogen cycle by increasing the ratio of fungi and bacteria and reducing the biocrust (Delgado et al., 2014). Whittington et al. (2013) found that warming indirectly affected the nitrogen cycle by changing the biomass and material composition of legumes in a prairie in North America. Wang et al. applied an undisturbed soil column grafting from meadows with different altitudes to transplant high altitude of undisturbed soil column to low altitudes along Xilingol Basin. One growing season incubation experiment showed that net nitrogen mineralization rate increased in soil migrated to low altitudes, which implied that climate warming would lead to soil nitrogen mineralization rate increase in the alpine meadows. Luo et al. found that warming accelerated the decomposition of soil organic matter and improved the utilization rate of soil nutrients through a short-term warming experiment in a tallgrass prairie in the North America. Zhang et al. (2014b) found that warming reduced orthophosphate but increased monophosphate and reduced the recovery of soil phosphorus through P-31 NMR method through a short-term warming experiment in a temperate grassland, implying that future climate warming would affect the soil phosphorus cycle.

1.2.4 Effects of warming on soil respiration

Soil respiration is mainly composed of autotrophic respiration and heterotrophic respiration (Hanson et al., 2000). Autotrophic respiration includes root respiration and rhizosphere microbial respiration, while heterotrophic respiration mainly refers to degradation of organic matter and respiration of soil animals and microorganisms (Kuzyakov, 2002). The substrates of autotrophic respiration are different from heterotrophic respiration. The substrates of autotrophic respiration are derived from the photosynthetic products distributed to the belowground part of plants, while the substrates of heterotrophic respiration are mainly from soil organic matter. As an important way of carbon exchange between soil and atmosphere, soil respiration plays an increasingly important role in global carbon cycle with the aggravation of global warming. Therefore, climate warming research has attracted more and more attention in recent years.

Due to the wide distribution of grassland ecosystem, the response of soil respiration in grassland ecosystem to warming is spatially heterogeneous. For example, Reynolds et al. (2015) conducted a warming manipulative experiment in three American savannahs and found that, through a 18-month soil respiration measurement, warming stimulated soil respiration, specifically total annual respiration in the northern station with higher monthly mean temperature increased by 28.6% and that in the central station with lower monthly mean temperature increased by 13.5%. Zhao et al. (2019) conducted a 2-year warming manipulative experiment in a semi-arid grassland in Inner Mongolia and found that warming had an inhibitory effect on the soil respiration mainly due to the reduction in microbial biomass carbon and changes in plant biomass. Liu et al. (2009) conducted a 3-year field warming manipulative experiment in a semi-arid temperate grassland and found that warming reduced soil respiration by decreasing soil moisture. Therefore, to understand the spatial and temporal variations of soil respiration rate and its biotic and abiotic drivers is of great scientific significance for tackling climate change.

The temperature sensitivity index (Q_{10}) of soil respiration, as an indicator of the response of soil carbon pool to climate warming, has been widely used in earth process models (Davidson and Janssens, 2006). It represents the multiple increase of soil respiration rate with each temperature increase of 10 ^{o}C. In the early earth process models, most researchers used a single soil respiration temperature sensitivity index to predict soil respiration and temperature changes, and further predicted global temperature changes on this basis. However, Q_{10} is not only affected by temperature, but also regulated by soil moisture, vegetation type, regional climate, soil chemical properties and microbial activity. Therefore, there may be different Q_{10} values in different environments. Long-term warming reduced the temperature sensitivity of soil respiration, and therefore predicting that either the magnitude of warming or the duration of warming would significantly affect the temperature sensitivity of soil respiration (Luo et al. 2001). This important finding is the now widely studied adaptation of soil respiration to temperature.

Grassland ecosystems are distributed from arid to humid, from temperate to cold zones, so the temperature sensitivity of warming to soil respiration shows regional differences. Carey et al. (2016) analyzed the Q_{10} data in global terrestrial ecosystems and found that warming did not change the Q_{10} of soil respiration in grassland ecosystems. Ding et al. (2016) also found that there was no significant correlation between the Q_{10} of soil respiration and temperature in an alpine grassland and an alpine meadow in the Qinghai-Tibet Plateau. However, by analyzing the Q_{10} of soil respiration in five typical grassland ecosystems, Feng et al. (2018) found that the Q_{10} of soil respiration decreased with the increase of temperature. Thus, appropriate Q_{10} value according to different regions should be taken into the simulation studies of grassland ecosystem carbon cycle using ecosystem process model for more accurately predicting the responses of ecosystem carbon cycle to climate warming.

1.3 Effects of nitrogen addition on grassland ecosystems

1.3.1 Effects of nitrogen addition on plant growth

Nitrogen is a limiting factor affecting the sustainable development of grassland ecosystem (Leblans et al., 2014; Liu et al., 2011; Ma et al., 2020; Ren et al., 2017; Song et al., 2019; Xu et al., 2017). Nitrogen addition could increase the grassland ecosystem productivity. For example, Pan et al. (2005) found that nitrogen addition significantly increased the density, height, aboveground biomass, belowground biomass and total biomass of Leymus chinensis in a typical grassland in Inner Mongolia. With the increase of nitrogen addition level, the plant aboveground biomass also increased significantly (Li et al., 2009; Bai et al., 2010), which is related to soil nitrogen condition due to the increase of soil nitrogen availability and reduce the limit of nitrogen (Pan et al., 2004; Bai et al., 2010). In addition, nitrogen addition can improve nitrogen, chlorophyll content and specific leaf area (Wan et al., 2008; Huang et al., 2009). Nitrogen addition also alters the productivity and competitive balance of C3 and C4 plants. For example, Niu et al. (2008) found that the increase in biomass of T. sinensis was significantly higher than that of T. sinensis, indicating that T. sinensis was less affected by nitrogen addition than T. sinensis. Thus, the effects of nitrogen addition on plant growth in grassland ecosystems depend on both biotic and abiotic factors. Therefore, elucidating the biotic and abiotic driving mechanisms of plant growth in grassland ecosystems in response to atmospheric nitrogen deposition is of great scientific significance for accurately predicting the impacts of future global changes on the global carbon cycle.

1.3.2 Effects of nitrogen addition on plant and microbial communities

The increasing atmospheric nitrogen deposition is one of the important driving factors for the change of ecosystem community structure (Liu et al., 2011). Nitrogen addition could reduce the species diversity of grassland ecosystem. For example, Stevens et al. (2004) found that long-term increased nitrogen deposition significantly reduced plant species richness by synthesizing the data from 68 acidified grasslands in the UK. Li et al. (2009) found that with the increase of nitrogen addition level, the species richness and diversity in the study area decreased significantly. Different plant species have different nitrogen utilization efficiency, and nitrogen addition could change the structure of plant community by increasing the dominance of a few dominant plants in the plant community. Bai et al. (2010) found that nitrogen addition caused the decrease of biodiversity and species richness by increasing the dominance of annual plants and decreasing the dominance of perennial and non-grasses. The response of soil microbial community in grassland ecosystem to nitrogen addition exists threshold. Zhang et al. (2008) found that the microbial diversity significantly decreased when nitrogen was added at a high dose, but did not change significantly when nitrogen was added at a low dose in a semi-arid temperate grassland. The decrease of soil pH caused by medium level nitrogen addition may be the main reason for the decrease of microbial diversity. Zhang et al. (2008) also suggested that the nitrogen addition load in the temperate grassland was between 160-320 kg N· ha^{-1} · yr^{-1}, and the microbial community would differently respond when exceeding this nitrogen load.

1.3.3 Effects of nitrogen addition on soil chemical properties

Nitrogen in the soil is mainly in the form of organic nitrogen, which usually accounts for more than 90% of the total nitrogen in the topsoil, and inorganic nitrogen in the soil accounts for 1%-3% of the total nitrogen. Atmospheric nitrogen deposition is an important component of the global nitrogen cycle. The increase of nitrogen deposition can significantly affect the nitrogen conversion process (nitrogen mineralization rate and nitrification). In grassland ecosystem, nitrogen addition could usually improve the nitrogen availability, enhance microbial activity and promote the mineralization of organic nitrogen in soil. Studies have shown that nitrogen addition can significantly improve soil inorganic nitrogen (NH_4^+-N and NO_3^--N) and net nitrogen mineralization rate and promote nitrification, but reduce soil microbial carbon. Through a 3-year nitrogen addition (0-640 kg N· ha^{-1} · yr^{-1}) experiment, Zhang et al. (2008) found that soil NH_4^+-N, NO_3^--N, net nitrogen mineralization rate and nitration rate increased in the growing season year by year. Zhang et al. (2009) found that low nitrogen addition (17.5 kg N· ha^{-1} · yr^{-1}) promoted nitrogen mineralization, whereas high nitrogen addition (280 kg N· ha^{-1} · yr^{-1}) inhibited nitrogen mineralization. However, long-term nitrogen addition also reduced soil pH value, leading to decreased microbial activity, decomposition of soil organic matter and mineralization of organic nitrogen, resulting in soil nutrient imbalance (Wang et al., 2019; Zeng et al., 2018).

1.3.4 Effects of nitrogen addition on soil respiration

Nitrogen addition does not directly affect soil respiration, but indirectly affects soil respiration by changing soil chemical characteristics, affecting plant growth, and changing microbial activity. In grassland ecosystems, short-term nitrogen addition can effectively improve soil fertility, promote the growth of plants and microorganisms, and thus improve soil respiration. With the accumulation of nitrogen, the soil pH could decrease, and the increase of soil H^+ has toxic effects on the cell wall of microorganisms, thus inhibiting the growth of microorganisms and reducing the microbial enzymes activity. Then microorganisms and plants will not have sufficient substrate supply, which will lead to the inhibition of soil respiration (Chen et al., 2016a; Zeng et al., 2018).

1.4 The basis for project approval, scientific issues, scientific objectives and research contents

1.4.1 The basis for project approval

A series of important ecological problems caused by climate warming and atmospheric nitrogen deposition have become the focus of current research. They have a great impact on the biodiversity, community structure and biogeochemical cycling process of the ecosystem, especially the grassland ecosystem. At the same time, the ecosystem will produce positive or negative feedback.

Grassland, which accounts for about 40% of the world's land area, is one of the world's largest vegetation types and is vital to food security, such as the supply of ruminant animal feed. Grassland ecosystem plays an important role in the balance of greenhouse gases, especially in global carbon stocks and carbon sequestration. As a key factor driving ecological processes, temperature affects almost all biotic and abiotic processes in an ecosystem. As a common limitation element in natural ecosystems, the increase of available nitrogen will reduce the lack of nitrogen, while atmospheric nitrogen deposition is the only source of inorganic nitrogen in natural ecosystems. The ecosystems with high latitudes or high elevation in the northern hemisphere are sensitive to climate change. Therefore, it is of great significance to investigate the effects of climate warming and atmospheric nitrogen deposition on grassland ecosystems in these regions.

1.4.2 Scientific issues and objectives

Rising temperature and/or nitrogen deposition can substantially influence the global carbon budget, consequently leading to positive or negative feedback to climate change (Janssens et al., 2010; Luo et al., 2001; Melillo et al., 2002; Niu et al., 2010; Rustad et al., 2001). Soil, which contains a pool of carbon approximately double that stored in the terrestrial biomass (Schlesinger and Andrews, 2000), plays a vital role in the global carbon cycle. A 10% loss of soil organic carbon in soil amounts to 30 years' worth of all the anthropogenically emitted atmospheric carbon dioxide (CO_2) (Kirschbaum, 2000). Soil respiration (Rs) is the largest carbon efflux from soil to the atmosphere (Schimel, 1995), and changes in Rs could either slow down or speed up the elevation of atmospheric CO_2 (Luo et al., 2009; Tu et al., 2010; Wang et al., 2014; Zhou et al., 2016). Numerous manipulative experiments conducted over the past two decades have demonstrated that the effects of warming and/or nitrogen addition on Rs can be complex. Previous studies have found that warming led to a persistent increase (Hicks Pries et al., 2015; Xu et al., 2015; Zhou et al., 2007), an initial increase and then attenuated after only a few years (Rustad et al., 2001) and a decrease (Liu et al., 2009; Reynolds et al., 2015; Saleska et al., 1999) in Rs. Increasing nitrogen deposition also resulted in different effects on Rs, including stimulation (Graham et al., 2014; Tu et al., 2010; Yan et al., 2009; Zhang et al., 2014), inhibition (Bowden et al., 2004; Janssens et al., 2010; Maaroufi et al., 2015; Olsson et al., 2005; Tao et al., 2013; Yan et al., 2009) or only slight effects (Allison et al., 2008; Lee and Jose, 2003; Maaroufi et al., 2015; Mo et al., 2007; Zhang et al., 2014a; Zhu et al., 2015). The apparent inconsistency of these responses can be attributed to interactions between biotic and abiotic factors, including microbial carbon-use efficiency (Frey et al., 2013; Tu et al., 2010; Zhang et al., 2014a), substrate supply (Melillo et al., 2002) and nitrogen and soil water availability (Contosta et al., 2011; Liu et al., 2009; Reynolds et al., 2015; Tao et al., 2013; Wan et al., 2007; Yan et al., 2016). Rs can be mainly contributed by roots and rhizosphere microbes (i.e. autotrophic respiration) in the growing season but likely comes from saprotrophic microorganisms (i.e. heterotrophic respiration) in the non-growing season (Schindlbacher et al., 2009; Schmidt et al., 2004; Zhou et al., 2007). However, most investigations of warming and nitrogen fertilizer have not explicitly addressed the seasonal (growing or non-growing season) difference of Rs responses to warming and nitrogen deposition.

Soil respiration is a combination of soil autotrophic respiration (Ra) and heterotrophic respiration (Rh) and they might respond differently to environmental changes, which increases the difficulty to evaluate the role of soil respiration in global C cycling under global change. Simulated warming in a meadow grassland reduced Rh but had no effect on Ra (Chen et al., 2016b), while in an alpine meadow ecosystem, it enhanced rates of both Rh and Ra (Peng et al., 2015). Differential responses for Rh and Ra were also reported for N experiments. A meta-analysis suggested that N deposition stimulated Ra but inhibited Rh in global grasslands (Zhou et al., 2014). It is clear that understanding how each component, Rh and Ra, responds to environmental drivers is essential in order to develop a comprehensive understanding of the role of Rs in the global C cycle under global change.

Even though soil microbial community is the engine responsible for this SOM decomposition (Ali et al., 2018; Cheng et al., 2017; Frostegård and Bååth, 1996; Zhou et al., 2016), a general conclusion as to how soil microbial community composition will be affected by warming remains elusive (Radujkovic et al., 2018). Therefore, knowledge about the warming responses of microbial communities is still needed to better understand global C cycling in the future warmer climate.

This is one of the most important questions still unanswered today in how northern ecosystems will respond to future climate warming. Aboveground litter fall and fine and coarse root turnover contribute the primary inputs of organic matter into soil, although root exudates and mycorrhizae also represent potential soil carbon inputs (Godbold et al., 2006; Ven et al., 2019; Vicca et al., 2012). Therefore, understanding the distribution of above- and belowground biomass and production to projected climate warming is crucial for both empirical studies and future ecosystem carbon model development. In high latitude ecosystems, the belowground processes are often relatively more important in the ecosystem carbon balance than the aboveground litter fall (Semchenko et al., 2018; Walker et al., 2018). However, knowledge on root systems is very limited in comparison to the much better understood aboveground production (Kong et al., 2019; Qi et al., 2019; See et al., 2019).

Thus, we put forwarded the scientific issues as follows based on above contents:

(1) Whether does soil respiration seasonally respond to warming and nitrogen addition?

(2) How will soil heterotrophic and autotrophic respiration respond to warming and nitrogen addition?

(3) How will soil microbial communities respond to warming?

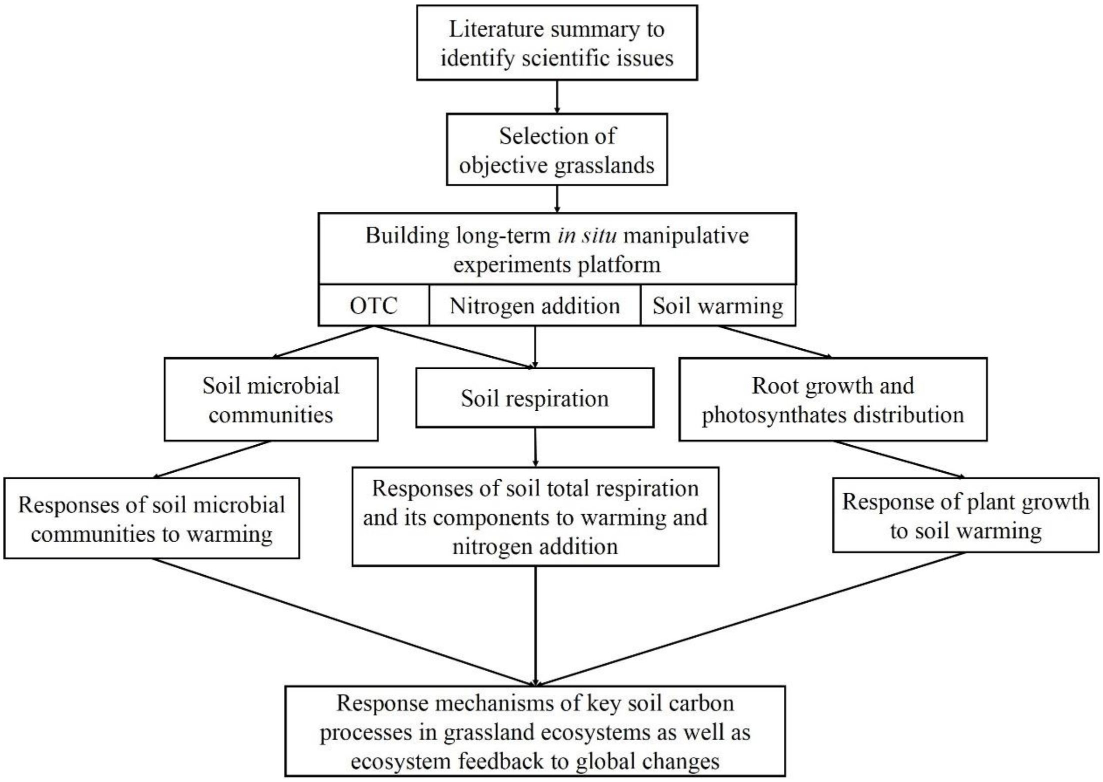
(4) How will plant root growth and photosynthates distribution respond to warming?

In view of the above scientific issues, field manipulative experiments were conducted in a typical alfalfa grassland and a fence natural grassland in the semi-arid region of loess Plateau to 1) explore the seasonal changes of soil respiration in semi-arid grassland ecosystem under warming and increased nitrogen deposition; 2) master the driving factors of responses of its components to warming and nitrogen addition; 3) elucidate the biotic and abiotic driving pathways of soil microbial community in response to warming. Meanwhile, a long-term soil warming experiment was carried out in a sub-arctic grassland ecosystem to explore the response of plant growth in a subarctic grassland ecosystem to climate warming. These field manipulative experiments are expected to provide important parameters and model validation for simulation of soil key carbon processes in climate-sensitive grassland ecosystems and terrestrial ecosystem processes in response to climate change.

1.4.3 Research contents

In this paper, alfalfa grassland and fenced natural grassland in the semi-arid area of loess Plateau and sub-arctic region were study objectives. In the alfalfa grassland, a completely random design was adopted. In the fence grassland, a random blocking experiment was designed. We used open-top chambers to simulate temperature increase, ammonium nitrate solution to simulate nitrogen deposition increase, and micro-trench method to distinguish soil autotrophic respiration and heterotrophic respiration. In the subarctic grassland ecosystem, five transects were established with a natural soil temperature gradient caused by the earthquake. Thus, a long-term field *in situ* manipulative experiment platform was established under the conditions of climate warming and increased atmospheric nitrogen deposition. The main research contents include: 1) analyzing the seasonal dynamics of soil respiration in the alfalfa grassland under temperature increase and nitrogen addition; 2) quantifying the response of soil respiration and its components to temperature increase and nitrogen addition as well as its regulatory factors; 3) to compare the responses and mechanisms of different microbial communities to temperature increase; 4) to explore the driving mechanisms of plant root growth and photosynthate distribution in response to long-term soil temperature increase.

1.5 Framework of technical line



Chapter 2 Seasonal responses of soil respiration to warming and nitrogen addition in a semi-arid alfalfa-pasture of the Loess Plateau, China

The Loess Plateau of Northwestern China is one of the most eroded regions in the world (Chen et al., 2007; Turner et al., 2011), and plays an important role in the global biogeochemical carbon cycle and climate change research (Shi et al., 2011; Ueyama et al., 2009). The semi-arid area as a key region in the Loess Plateau of China is characterised by low precipitation, soil water content and vegetation cover as well as severe soil erosion (Chen et al., 2007; Gao et al., 2009; Ye et al., 2013; Zhang, 1989). Consequently, it is susceptible to climate change. In addition, concurrent changes in temperature and nitrogen deposition may potentially trigger complex interactive effect on R_S (Graham et al., 2014; Zhu et al., 2015). Although several studies have examined the effects of climate change on soil respiration in this region, they mainly focused on a single environmental driver, such as precipitation change (Gong et al., 2015; Jia et al., 2014; Wang et al., 2015) or nitrogen addition (Zhang et al., 2014). To the best of our knowledge, no detailed investigation of the interactive effect of warming and nitrogen addition on soil respiration in this region has been conducted.

In order to evaluate the effects of warming and nitrogen addition on R_S, we conducted a manipulative experiment on a typically semi-arid pasture in semi-arid region of Loess Plateau, China. The ecosystems in this region are susceptible to environment changes. We hypothesised that (1) warming and nitrogen addition may have positive effects on R_S through interaction, because temperature and nitrogen could positively affect available nitrogen which is main growth factor of plant and microbe; (2) drought stress may negate the superficially positive effect of warming or nitrogen because water limitation can inhibit plant growth or microbial activity; and (3) R_S may respond to warming and nitrogen with seasonal heterogeneity, considering seasonal discrepancy of R_S biologically drivers (i.e. growing season and non-growing season). In addition, as this study was carried out in wet year (2014) and dry year (2015), water variations might influence the potential responses of R_S to warming and nitrogen addition.

2.1 Materials and methods

2.1.1 Study area

The study was conducted from 2014 to 2016 at the Semiarid Ecosystem Research Station on Loess Plateau (36°02′N, 104°25′E, 2400 m above sea level), Lanzhou University. The station is located in Yuzhong County, Gansu Province, China (Fig. 2.1). The site has a medium-temperate semi-arid climate. Mean annual precipitation is 305 mm and varies greatly annually, 80% of which occurs in the growing season (April to October). Mean annual pan evaporation is about 1300 m. Mean annual air temperature is 6.5 °C from 1955–2013, and mean growing and non-growing (November to March) season temperatures are 11.7 °C and –5.3 °C, respectively. The soil is Heima soil (Calcic Kastanozem, FAO Taxonomy) with high percentage of silt (Table 2.1). The study area was sown with *Medicago sativa L.* in April 2003 and fenced without grazing, tillage, fertilisation, harvesting or any other management then. Plantation of the legume species aimed to facilitate the revegetation of degraded land thus improving vegetation cover and reduce soil erosion in this region (Yuan et al. 2016). The plant community at the study site is dominated by *M. sativa L.*, *Heteropappus altaicus Novopokr.*, *Stipa breviflora Griseb.*, *Artemisia capillaris*, *Leymus secalinus Tzvel.*, and *Androsace ereta Maxim.* during experimental period.

Table 2.1 Soil properties of 0-20cm profile in the study site (measured in May, 2013)

Soil Property*	Bulk density (g cm^{-3})	clay (%)	silt (%)	sand (%)	Soil organic carbon (g kg^{-1})	Soil total nitrogen (g kg^{-1})	Soil total phosphorus (g kg^{-1})
Value	1.22	9	78	13	7.8	0.93	0.19

*Soil bulk density was measured using cutting-ring method; soil particle size was measured according to the FAO taxonomy; soil organic C was determined with the Walkley-Black method; soil total nitrogen was determined with the K_2SO_4-$CuSO_4$-Se distillation method, and soil total phosphorus was determined with the molybdate colorimentric method after perchloric acid digestion and ascorbic acid reduction.

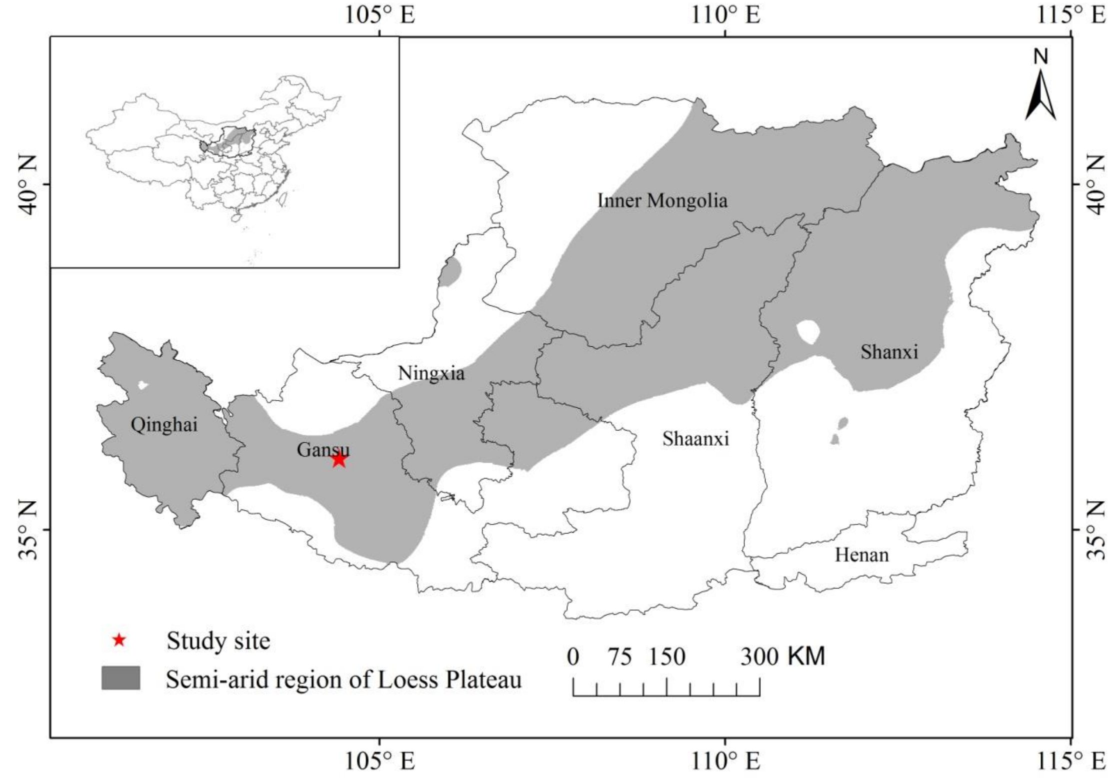


Fig. 2.1 Geographic location of the study site

2.1.2 Experimental design

A completely random design was used in the experiment. Four treatments were included: control (Co), nitrogen addition (N), warming (W) and combined warming and nitrogen addition (WN). Each treatment had six plots (the plot area is 0.65 m^2), three of which were used for R_s measurement and the other three were used for vegetation coverage measurement. The open-top chambers (OCTs) used in the treatment plots was a hexagonal design with sloping sides of 40 cm×50 cm×32 cm (Maestre et al., 2013); the OCTs were placed on the soil surface and provided year-round warming to the enclosure.

Nitrogen was applied as ammonium nitrate (NH_4NO_3) at a rate of 4.42 g N m^{-2} y^{-1} in the N and WN treatments since March 2014. The total amount of nitrogen was equally divided into five applications in the early of April, May, June, July and August every year. The rate of nitrogen addition was chosen to represent twice as much the highest rate of atmospheric N deposition at this site estimated at about 2.21 g N m^{-2} y^{-1} (Lü and Tian, 2007). In each application, the fertilizer was dissolved in 65 mL of distilled water and applied to each plot with a sprayer; the control plots received 65 mL of distilled water. The total amount of water added to the plots by these treatments corresponded to 0.5 mm of extra precipitation each year.

2.1.3 Measurement protocols

Among the R_s measurement plots, a PVC collar (11 cm in diameter and 8cm in height) was inserted into the soil to a depth of 5 cm at the center of each plot for measuring soil CO_2 efflux. Since March 2014, CO_2 efflux was being measured, using a Li-8100 infrared gas analyzer (LI-COR Biosciences, Lincoln, Nebraska, USA). R_s was measured twice per month in growing season and once per month in the non-growing season during the study period. Living plants inside the PVC collars were clipped at the soil surface at least one day before measurements to eliminate the effect of aboveground biomass respiration. The clipped plant material was left inside the collars to be decomposed. To avoid any pulse effect of precipitation on R_s, measurement was performed at least three days after a rainfall event or a water addition treatment. All R_s measurements were made twice between 09:00 and 11:00 a.m. (local time). Each treatment took about 2-3 min.

To measure the vegetation coverage, we used a digital camera to vertically photograph every plot twice each month from May to September every year. The supervised classification approach (in ArcGIS software) was then applied to derive vegetation coverage from the digital photographs.

Given that nitrogen addition had no significant effect on air and soil moisture and temperature in the region (Zhang et al., 2014), we only measured these variables under warming and control treatments. One plot of warming treatment and one of control plot were randomly chosen for soil and air temperature and moisture measurement. The air temperature was recorded using HOBO® T/RH U23-002 data loggers (Onset Computer Corporation, Bourne, Massachusetts, USA). Soil temperature and volumetric soil moisture were recorded using Watchdog 1000 Series Micro station-T/RH (Spectrum Technologies Inc., Plainfield, IL, USA) at a depth of 5 cm. All these data were recorded at a 1 hour interval over the two years.

On the basis of the measured soil CO_2 efflux and soil temperature, the temperature sensitivity of R_s was calculated by fitting the exponential function (see Eq 2-1 below) in each individual treatment.

$$R_s = ae^{bT} \qquad 2\text{-}1$$

In the equation above, R_s is measured soil respiration (μmol m^{-2} s^{-1}), T is measured soil temperature (°C) at a depth of 5 cm, a is the basal R_s rate and b is a constant used to calculate the temperature sensitivity of R_s (Q10 value, the proportional rate of increase of SR when the soil temperature is increased by 10 °C; see Eq 2-2).

$$Q10 = e^{10b} \qquad 2\text{-}2$$

2.1.4 Leaf and soil sampling

*Medicago sativa L.*leaf samples were collected from all 12 plots for vegetation coverage measurement on 24 June in 2014. All leaves were oven-dried at 65 ^{o}C for 72 h. Soil samples were collected from the 12 plots with leaf samples on 24 June and 5 December in 2014. Soil samples were air-dried for the estimation of soil total nitrogen concentration. Soil and leaf total nitrogen concentrations (g kg^{-1}) , respectively, were measured using the Kjeldahl method.

2.1.5 Statistical analysis

Paired-t test was employed to compare the difference of soil and air temperature and moisture for the paired warming and control treatment. The three-way analysis of variance (ANOVA) was adopted to examine the main and interactive effects of warming, nitrogen addition and year on the mean annual R_s and Q10. Repeated-measures ANOVA tests were used to examine the effects of warming and nitrogen addition on R_s. Soil and leaf total nitrogen concentration, vegetation coverage and cumulative R_s (estimated by smoothly incrementing between monthly R_s across the number of days elapsed) were tested for treatment differences with the two-way ANOVAs. Significant differences were evaluated at the level $P<0.05$.

To determine the factors that drive the variation in R_s, exponential and linear regression analysis were conducted between R_s and soil temperature, soil moisture, and vegetation coverage. Stepwise multiple regression analysis was then used to ascertain the contribution of the above factors in affecting R_s. All statistical analysis were performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA).

2.2 Results

2.2.1 Microclimate

During the study period, precipitation showed great inter-annual variations, it was 392 mm in 2014 (wet year) and 290 mm in 2015 (dry year) (Fig. 2.2). Air and soil temperatures showed seasonal variation as that peaked on 29 July in 2014 and 27 July in 2015 in air and peaked on 2 August in 2014 and 31 July in 2015 in soil. Soil temperature peaked about 4 days later than that of air (Fig. 2.2). This is a phenomenon called “hysteresis”. Paired t-test results suggested that soil volumetric water decreased by 12% on average under W as compared with Co during January 1 in 2014 to March 9 in 2015 (i.e. non-growing season, Fig. 2.2). However, soil volumetric water significantly decreased by 20 % in the growing season (Fig. 2.3 A and B). On average, W increased soil and air temperatures by 0.7 °C and 1.6 °C, respectively, during the whole experimental period (Fig. 2.3).

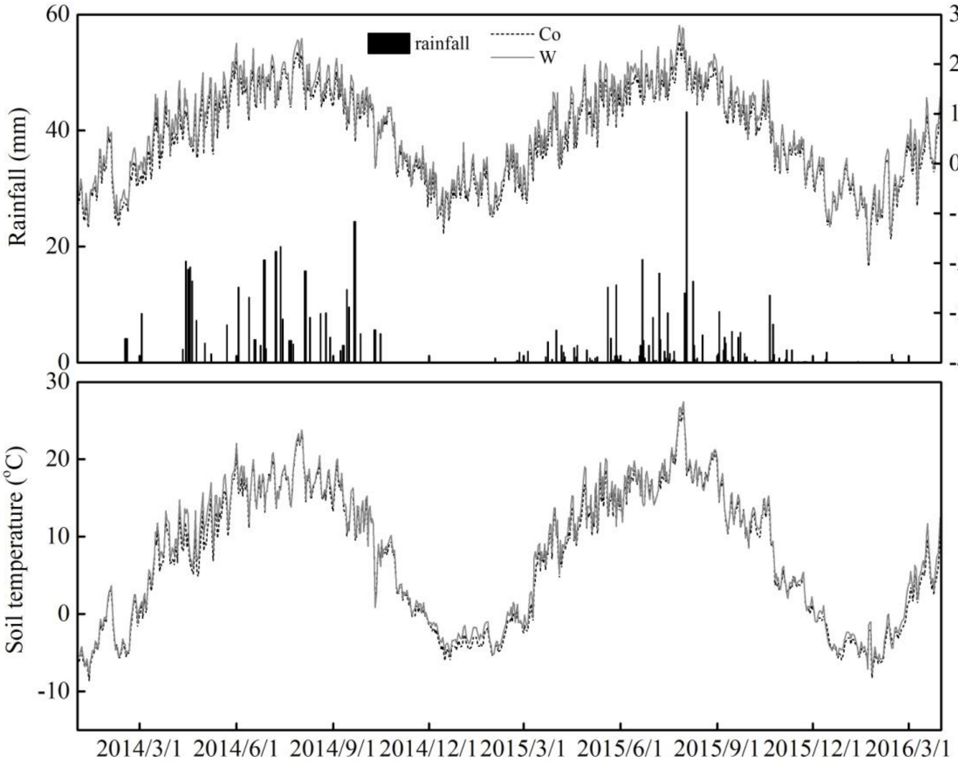


Fig. 2.2 Seasonal variations of air temperature, precipitation and soil temperature and moisture at 5 cm soil depth in the alfalfa stand pasture from January 2014 to March 2016.

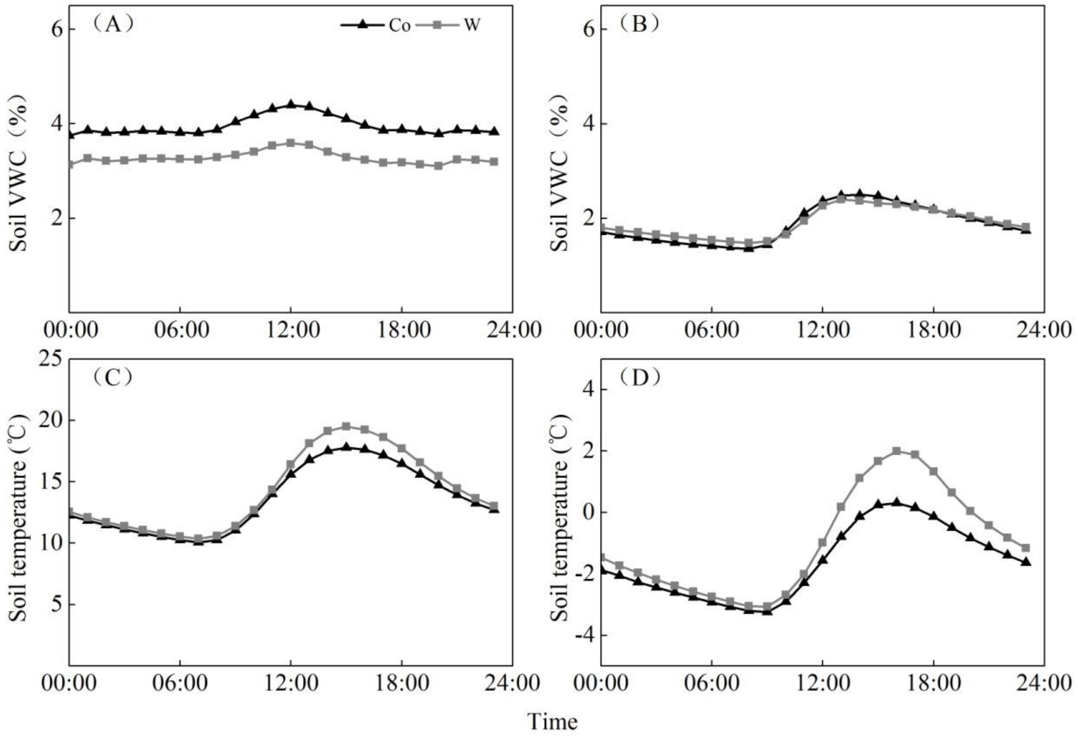


Fig. 2.3 Mean diurnal soil volumetric water content (VWC) during growing season (A) and non-growing season (B) and mean diurnal soil temperature during growing season (C) and non-growing season (D) in 2014. No soil moisture data were available from March 10 in 2015 to March 30 in 2016 due to instrument failure.

2.2.2 Variation in soil respiration

R_s showed similar seasonal dynamics as precipitation and air temperature, which peaked in the growing season (Figs. 2 and 4). The maximum R_s occurred in July of 2014 and August of 2015, and the minimum R_s in January and December of 2015. Nitrogen addition mainly increased seasonal variation of R_s by strengthening the maximum values whereas the opposite was observed after warming mainly by weakening the maximum values and strengthening the minimum values (Fig. 2.4).

Nitrogen addition significantly stimulated R_s during the two years (Fig. 2.4). Month interacted with N addition to affect R_s but there was no interactive effect between year and nitrogen addition on R_s (Table 2.2). Nitrogen addition alone enhanced R_s by 12% in 2014, 14% in 2015, 13% in the growing season of 2014, 14% in the growing season of 2015 and 57% in the non-growing season of 2015-2016. However, it did not significantly influence it in the non-growing season of 2014-2015. Nitrogen addition combined with warming stimulated average R_s by 18% in the non-growing season of 2014–2015 and 40% in the non-growing season of 2015-2016. Moreover, WN decreased average R_s by 0.5% in the growing season of 2014 and 2% in 2015, but had no significant influence on average R_s in 2014 and 2015 across the warming treatment (Fig. 2.4).

Warming significantly inhibited R_s during the two growing seasons (2014 and 2015), on the contrary, it significantly stimulated R_s during the two non-growing seasons (2014–2015 and 2015–2016) (Fig. 2.4). Significant interactions were observed between date and warming in affecting R_s (Table 2.2). When the data were analyzed for individual year using repeated measures ANOVAs, the main effect of warming was significant ($P<0.05$) over the two years (Table 2.2). Warming alone had no significant effect on the mean R_s in 2014, and in the growing and non-growing seasons of 2014-2015 (Fig. 2.4). Warming significantly inhibited annual and growing season R_s by 11% and 12%, respectively, whereas it stimulated R_s by 22% in the non-growing season of 2014. Warming significantly inhibited R_s by 8% and 9% in the annual and growing season of 2015, respectively, but stimulated it by 43% in the non-growing season of 2015-2016 ($P<0.05$). In comparison, the combined warming and N addition led to larger inhibition ($P<0.05$) of annual and growing season R_s in 2015 (19% and 21%, respectively), but less stimulation (27%, $P<0.05$) in the non-growing season of 2015-2016.

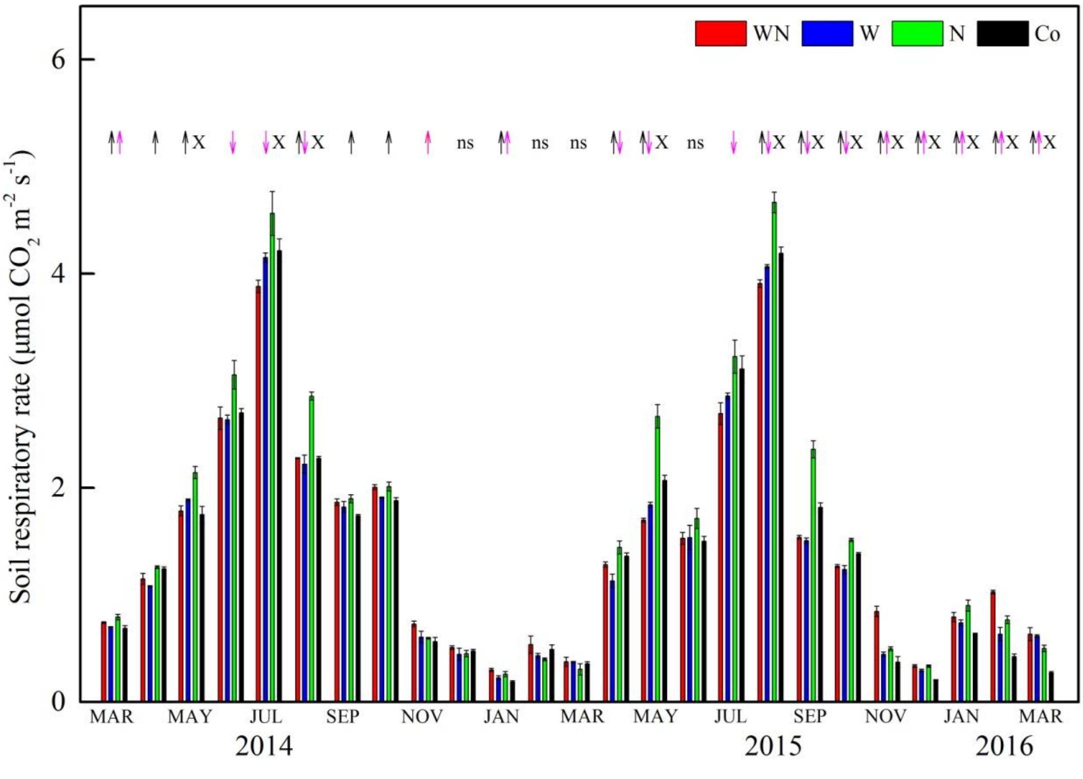


Fig. 2.4 Seasonal variation of soil respiration from March 2014 to March 2016 under four treatments. WN, combined warming and N addition; W, warming; N, N addition; Co, Control.

Upward arrows indicate significantly positive effect and downward arrows indicate significantly negative effect (*P*<0.05), nitrogen (black) and warming (pink); ns indicates non-significant (*P*>0.05). “X” indicates nitrogen and warming interactions.

Table 2.2 Results of repeated measures ANOVAs of the effects of Month or Year, Warming (W), N addition (N), and their interaction on soil respiration (μmol CO_2 m^{-2} s^{-1}) (n=3)

Year	Factor	*F*-Value	*P*
2014	Month	1790.89	<0.001
	Month ×Warming	8.40	<0.001
	Month ×N	2.72	<0.01
	Month ×Warming ×N	6.33	<0.001
	Warming	15.76	<0.01
	N	17.93	<0.01
	Warming ×N	13.64	<0.01
2015	Month	2113.06	<0.001
	Month ×Warming	27.64	<0.001
	Month ×N	4.07	<0.001
	Month ×Warming ×N	9.87	<0.001
	Warming	44.1	<0.001
	N	23.02	<0.001
	Warming ×N	8.86	0.018
2014-2015	Year	52.8	<0.001
	Year ×Warming	20.56	<0.01
	Year ×N	0.03	0.87
	Year ×Warming ×N	0.03	0.87
	Warming	48.91	<0.001
	N	20.31	<0.01
	Warming ×N	18.13	<0.01

2.2.3 Cumulative soil respiration

Over the two-year period, nitrogen addition significantly stimulated cumulative soil CO_2 efflux through R_s and this stimulation was not influenced by year (Fig. 2.5 A and B, Table 2.3). Cumulative R_s was 11% and 13% higher in N than in Co treatment in 2014 and 2015, respectively (Fig. 2.5 A and B). In the growing season, nitrogen addition significantly stimulated cumulative R_s by 13% in 2014 and 14% in 2015. In the non-growing season, nitrogen addition did not significantly influence cumulative R_s in 2014–2015 but significantly increased it by 45% in 2015–2016.

However, the response of cumulative R_s to warming varied in different years (Table 2.3). Specifically, warming did not affect the cumulative R_s in 2014 but significantly decreased it by 7% in 2015 (Fig. 2.5 A and B). Cumulative R_s in the growing seasons in two years showed similar patterns as the annual ones. Nevertheless, in the non-growing seasons, warming did not stimulate cumulative R_s in 2014–2015 but stimulated it by 30% in 2015–2016.

The combined effect of nitrogen addition and warming on cumulative R_s was significant ($P<0.01$) and it was not influenced by year (Table 2.3). Over the two-year period, cumulative R_s was significantly lower in WN than that in N throughout the whole year, however, no significant difference in the growing seasons under the two treatments were observed (Fig. 2.5 A, B, D and E). In the non-growing season, cumulative R_s was significantly higher in WN treatment than that in W and N treatments (Fig. 2.5 D and F).

Table 2.3 Results (*F*-Values) of repeated measures ANOVAs of the effects of Year, Warming (W), N addition (N), and their interaction on cumulative soil CO_2 efflux (g C m^{-2}) (n=3)

Year	Model	*F*	*P*
2014-2015	Year	68.28	<0.001
	Year ×Warming	10.94	<0.01
	Year ×N	0.65	0.445
	Year ×Warming ×N	0.05	0.829
	Warming	48.91	<0.001
	N	20.31	<0.001
	Warming ×N	18.13	<0.01

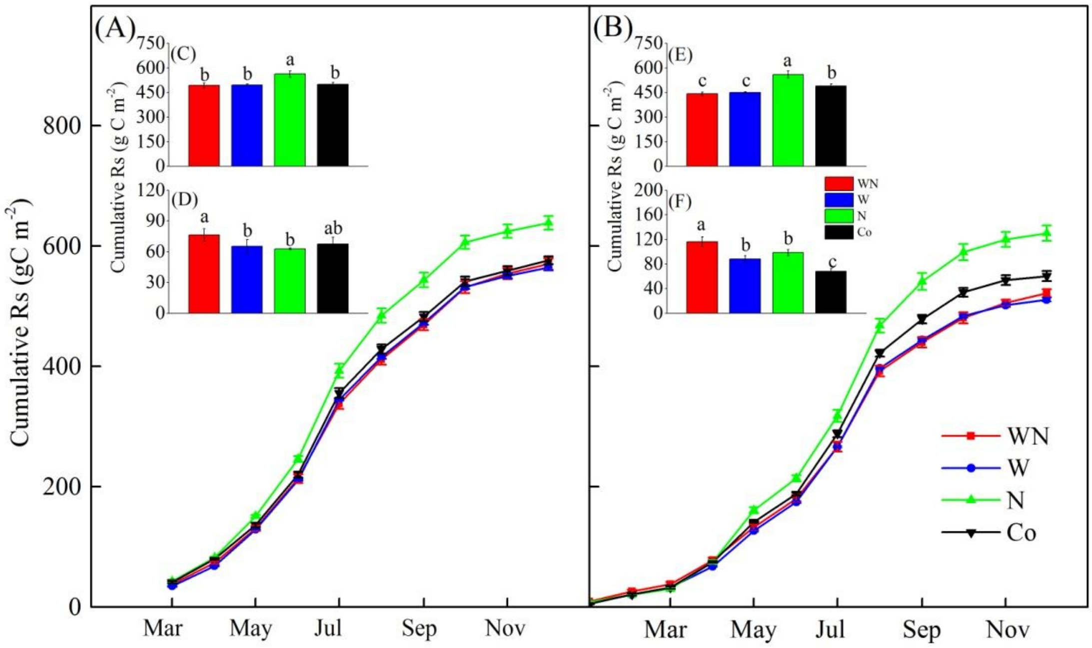


Fig. 2.5. Cumulative soil CO_2 efflux (R_s) from March to December 2014 (A) and from January to December 2015 (B) under the four treatments. Inserts show the cumulative CO2-C respired in growing seasons in 2014 (C) and 2015 (E) and non-growing seasons in 2014-2015 (D) and 2015-2016 (F) under combined warming and N addition (WN, red), warming (W, green), N addition (N, blue) and control (Co, black).

2.2.4 Temperature sensitivity of soil respiration

R_s was positively associated with soil temperature during the growing seasons and over the two-year period (Table 2.4). R^2 values indicated that the effects of temperature could explain 77%–85% variation of R_s over the two-year period and 45%–59% during the growing seasons (Table 2.4). Over the same period, Q10 in WN was significantly lower than that in the other treatments, whereas Q10 in W and N were significantly higher than those in Co and WN during the growing seasons (Table 2.4).

Table 2.4 Regressions equation between soil CO_2 efflux (R_s) and soil temperature in 5 cm soil depth (T) and the Q10 values (n=3)

Year	Treatments	Whole year	Growing season
2014	WN	$R_s=1.9e^{0.079T}$, $R^2=0.83^{***}$, Q10=2.20（0.05）b	$R_s=2.08e^{0.069T}$, $R^2=0.58^{**}$, Q10=2.01（0.10）ab
	W	$R_s=1.73e^{0.088T}$, $R^2=0.83^{***}$, Q10=2.44（0.14）a	$R_s=2.03e^{0.071T}$, $R^2=0.56^{**}$, Q10=2.03（0.05）a
	N	$R_s=1.81e^{0.91T}$, $R^2=0.84^{***}$, Q10=2.49（0.09）a	$R_s=2.23e^{0.072T}$, $R^2=0.59^{**}$, Q10=2.04（0.08）a
	Co	$R_s=1.82e^{0.083T}$, $R^2=0.80^{***}$, Q10=2.32（0.10）ab	$R_s=2.25e^{0.063T}$, $R^2=0.46^{**}$, Q10=1.89（0.06）b
2015	WN	$R_s=1.7e^{0.087T}$, $R^2=0.77^{***}$, Q10=2.40（0.14）b	$R_s=1.87e^{0.076T}$, $R^2=0.45^{**}$, Q10=2.12（0.03）b
	W	$R_s=1.55e^{0.1T}$, $R^2=0.8^{***}$, Q10=2.74（0.06）a	$R_s=1.74e^{0.085T}$, $R^2=0.49^{**}$, Q10=2.34（0.12）a
	N	$R_s=1.7e^{0.106T}$, $R^2=0.85^{***}$, Q10=2.90（0.14）a	$R_s=2.18e^{0.08T}$, $R^2=0.56^{**}$, Q10=2.22（0.05）ab
	Co	$R_s=1.63e^{0.103T}$, $R^2=0.79^{***}$, Q10=2.79（0.16）a	$R_s=2.06e^{0.076T}$, $R^2=0.49^{**}$, Q10=2.13（0.05）b

Different letters suggest significantly at P= 0.05,“* *”, P<0.01,“***”, P<0.001. The values in brackets were standard deviation.

2.2.5 Vegetation coverage and soil and leaf N

Over the two-year period, vegetation coverage showed similar seasonal dynamics with temperature and precipitation that peaked in July (Fig. 2.6). Warming significantly decreased the vegetation coverage ($P<0.05$) during the study period. Nitrogen addition alone or combined with warming did not significantly affect vegetation coverage ($P>0.05$).

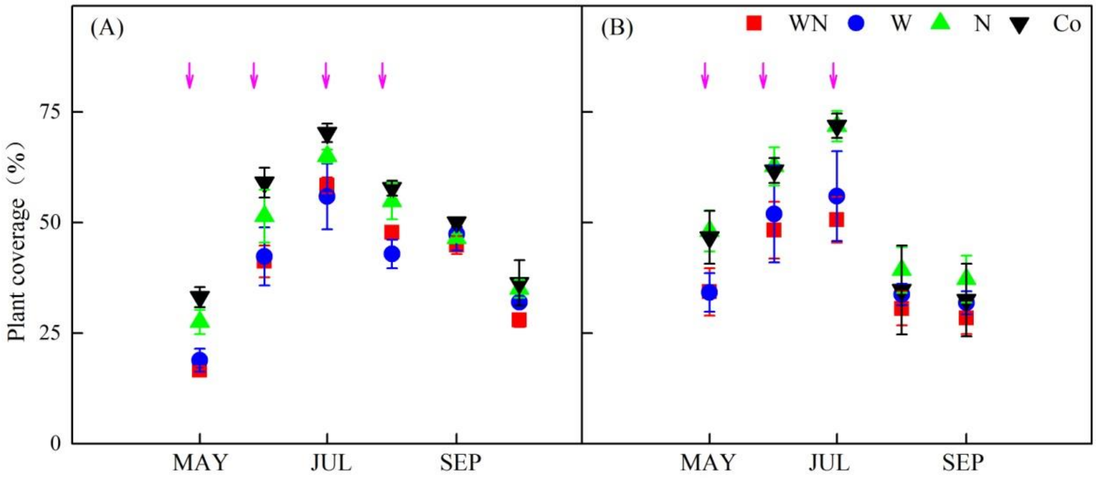


Fig. 2.6 Seasonal dynamics of vegetation coverage under the four treatments in 2014 (A) and 2015 (B). Downward arrows indicate significantly negative effect ($P<0.05$) due to warming (W). No significant effects were found under nitrogen addition alone (N) or combined with warming (WN).

In the growing season, soil TN under WN and W treatments were significantly higher than those under N and Co. However, no significant differences were observed between WN and W as well as between N and Co (Fig. 2.7 A). In the non-growing season, soil TN under WN and W treatments were significantly lower than that under Co, but there were no significant differences between WN and W as well as between N and Co (Fig. 2.7 B). No significant differences were observed in *Medicago sativa L.*leaf TN among the four treatments (Fig. 2.7 C).

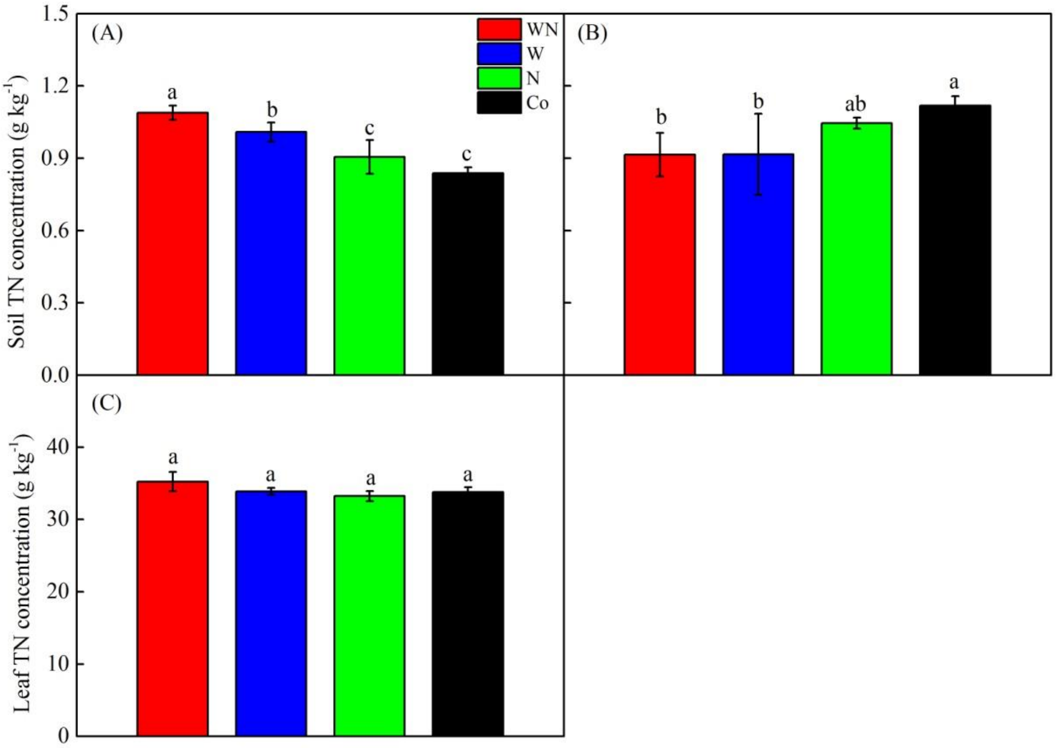


Fig. 2.7 Soil total nitrogen concentration (TN) of soil in June 24 (A) and December 5th 2014 (B) and *Medicago sativa L.*leaf TN in June 24th 2014 (C) under four experimental treatments. Combined warming and N addition (WN, red), warming (W, green), N addition (N, blue) and control (Co, black). Different letters suggest significantly at $P<0.05$.

2.2.6 Dependence of soil respiration on biotic and abiotic factors

When observations under all treatments were combined together, R_s increased exponentially with soil temperature ($R^2=0.0678$, $P<0.001$), and increased linearly with soil moisture and vegetation coverage ($R^2=0.202$ and 0.324, $P<0.001$, respectively, Fig. 2.8). Soil temperature could explain 72.5 % of the R_s variation in growing season but only 27.3 % in the non-growing season (Fig. 2.8 A). Soil moisture could explain 17.8 % of the R_s variation in growing season and 73.1 % in the non-growing season (Fig. 2.8 B). Step-wise multiple regression analyses with soil temperature, soil moisture and vegetation coverage as the predictors revealed that vegetation coverage ($R^2=0.682$) and soil moisture (Partial $R^2=0.040$) could explain 72.2 % of the variation in R_s.

The variabilities of R_s explained by soil temperature and moisture were compared for warming and control treatments. The Pearson's correlation coefficient of R_s vs. soil temperature under W ($R=0.636$, $P<0.05$) was lower than that under Co ($R= 0.641$, $P<0.05$) in the growing season, but it was higher under W ($R=0.900$, $P<0.01$) than that under Co ($R=0.842$, $P<0.01$) in the non-growing season. Soil moisture could not explain the variation in R_S (P>0.05) under either W or Co treatment.

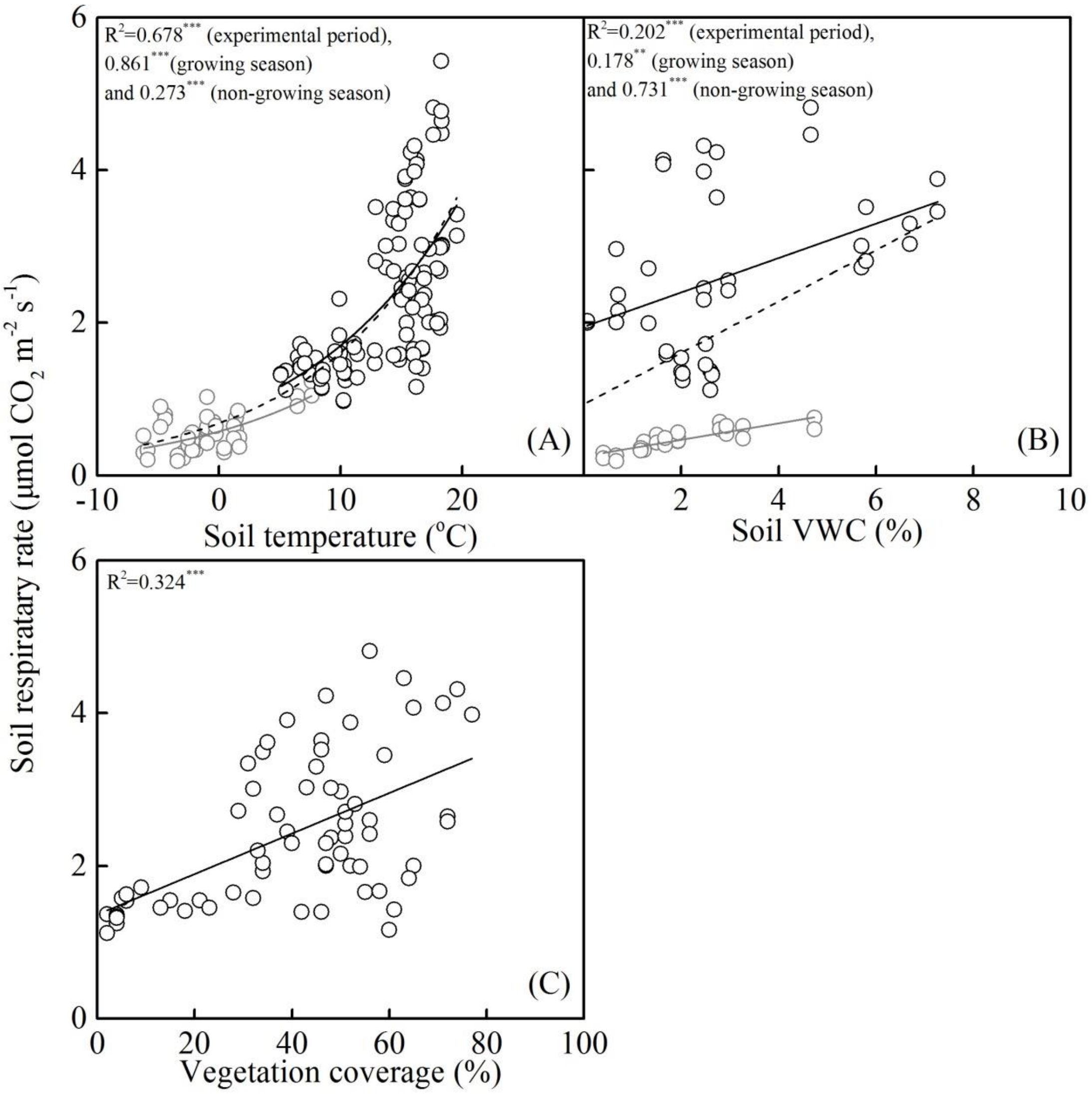


Fig. 2.8 Dependence of soil respiration on soil temperature, soil moisture and vegetation coverage across the experimental period (dashed line), in growing season (black solid line) and non-growing season (gray solid line). Black and gray circle mean growing season and non-growing season data, respectively. Each point represents each observation (the mean value of three replicates) during the two years (A) and (C), and from January 1 in 2014 to March 9 in 2015 (B). "**" and "***" indicates significant difference at P<0.01 and P<0.001.

2.3 Discussion

2.3.1 Seasonal variability

The large seasonal variation in R_S (Fig. 2.4) indicates that the alfalfa-pasture is sensitive to environmental change. This can be reflected by the similar seasonal changes of temperature or precipitation and R_S (Figs. 2 and 4), which suggest that the temperature and precipitation are important factors controlling R_S in the study site. The strong seasonal variability in R_s has also been reported in many other grassland ecosystems such as a tall-grass prairie (Zhou et al., 2007), a temperate steppe (Yan et al., 2009) and a stipa grassland (Zhang et al., 2015).

2.3.2 Nitrogen effect

Our study suggested that nitrogen addition generally stimulated R_s, which induced more CO_2 from soil into the atmosphere (Fig. 2.4). This finding agrees with several studies that nitrogen addition led to short-term increases in R_s (Comeau et al., 2016; Graham et al., 2014; Tu et al., 2010; Zhang et al., 2014), which may result from higher microbial utilization rate in labile carbon (Aber et al., 1998), accelerated microorganism access to plant litter (Craine et al., 2007) and/or increased plant belowground allocation (Bowden et al., 2004; Tu et al., 2010). In our study, nitrogen addition did not increase soil and leaf TN as compared with control plot in both the growing and non-growing seasons (Fig. 2.7), indicating that the microbial utilization of soil nitrogen increased under nitrogen addition treatment. Whereas in the long-term studies, a significant decrease in soil respiration was typically observed, which could be due to soil acidification (Chen et al., 2016), depletion of labile carbon (Frey et al., 2004), reduced microbial biomass (Chen et al., 2016), and/or reduced root biomass (Bowden et al., 2004). The stimulation of soil respiration in nitrogen addition treatment that we observed likely represents the initial phase of response, which may be followed by decline in longer-term. Meanwhile, the stimulation effect of nitrogen addition was not influenced by year in short-term (Table 2.2). This result indicated that R_s may not be able to adapt to nitrogen enrichment in the short-term.

2.3.3 Warming effect

Many warming experiments have reported either no change (Liu et al., 2015; Luo et al., 2001; Wan et al., 2007), increased (Zhu et al., 2015) or decreased (Saleska et al., 1999) in R_s after warming throughout the whole year. In contrast to these results, we found a negative effect of warming on R_s in the growing season and a positive effect in the non-growing season (Fig. 2.4). This difference could be explained by the changes in soil water and vegetation coverage. The positive effect of warming existed only in the non-growing season when warming did not reduce soil water content. Some previous studies have suggested that reduction in soil volumetric water content induced by warming may offset or even override the positive effects of soil warming on R_s (Graham et al., 2014; Reynolds et al., 2015). Soil water stress could not only directly suppress respiration by decreasing microbial and root activities, but also indirectly decrease R_s via inhibition of plant growth, below-ground carbon allocation, and substrate availability (Wan et al., 2007). In our study, warming decreased soil TN in the non-growing season, but it increased it in the growing season (Fig. 2.7 A and B), which may indicate that warming decreased the utilization of soil nitrogen in the growing season. Likewise, decline in plant coverage may indicate less total photosynthetic carbon, which could then lead to less amount channeling belowground, consequently reducing root respiration (Xu et al., 2015). In this study, we found a significantly positive correlation between soil water content and vegetation coverage (Fig. S1). Warming induced soil drying and thus reduced plant coverage in the growing season (Figs. 3 and 6), which may inhibit the positive effects of warming. Several previous studies have reported that in semi-arid conditions, warming-induced water stress often limited R_s by limiting microbial activities and/or root activity (Sheik et al., 2011; Wan et al., 2007). Other warming experiments, including those in forests, meadows or grasslands have reported that warming increased R_s by increasing microbial activity and root activity; however, in these mesic environments, warming did not significantly decrease soil moisture (Melillo et al., 2002; Peng et al., 2015; Wan et al., 2007). In our study, daily temperature was still below freezing under warming plots in the non-growing season (Fig. 2.2), the average 0.4 °C increase of soil temperature induced by warming did not lead to increase in soil evaporation and consequently did not change the soil moisture (Fig. 2.3 B). Increased soil temperature might promote microbial activity, which contributed more respiration by increasing C substrate decomposition rate. Therefore, the Pearson's correlation coefficient of Rs vs. soil temperature under W was lower than that under Co in the growing season, but it was higher under W than that under Co in the non-growing season.

Warming decreased annual cumulative R_s in the dry year but it was not altered in the wet year (Fig. 2.5 A and B). One possible explanation is that the different drought degree over the two years. The plant may suffer higher drought stress induced by warming in the dry year than that in the wet year, which resulted in less CO_2 efflux from soil into the atmosphere. An overview study on the response of R_s to drought stress reported that in a dry environment, R_s decreased in pace with increasing drought stress (Wang et al., 2013). Meanwhile, annual cumulative R_s in the wet year was significantly higher than that in the dry year (Fig. 2.5 A and B). Therefore, extreme drought event could regulate the response of soil carbon to warming in semi-arid region.

2.3.4 Interactions between warming and nitrogen addition

Interaction between two factors is considered as synergism, antagonism or addition. It is identified as synergism or antagonism when their combined effect is significantly stronger or weaker than the sum of the two individual effects, while it is considered additive interaction when the combined effect is not significantly different from the sum (Zhou et al., 2016). In our study, we observed antagonism between warming and nitrogen on R_s in the growing season and synergism in the non-growing season (Fig. 2.5). That antagonism occurred in the growing season, which corresponds well with the negative effect of warming on R_s, indicated an offset effect of warming on the nitrogen treatment. A similar result was observed in the Harvard Forest (Contosta et al., 2011), where a negative but marginally significant interaction between warming and nitrogen fertilization on R_s occurred in autumn. In comparison, soil water content did not change in the non-growing season, so we could ignore the negative effect of drought stress on R_s. Consequently, synergism between warming and nitrogen addition happened in the non-growing season, which indicated that warming synergistically interacted with nitrogen addition to accelerate R_s in the non-growing season. Therefore, the combined effect of nitrogen addition and warming may be regulated by soil water condition.

2.3.5 Temperature sensitivity of soil respiration

Thermal acclimation of R_s has been widely discussed in recent years. Previous empirical and modelling studies suggested that thermal acclimation could be due to the depletion of fast carbon cycling, physiological acclimation of microbes or plant and/ or changes in microbial or plant community composition (Luo et al., 2009; Luo et al., 2001; Shen et al., 2009; Xu et al., 2015). Q10 is considered one of the most important parameters to assess thermal acclimation of R_s (Luo et al., 2001; Raich and Schlesinger, 1992; Zhou et al., 2007). The Q10 values (ranged from 2.2–2.9) in our study are within the range (1.75–3.00) reported by previous studies in the semi-arid region of Loess Plateau (Zhang et al., 2015) and the global average value (i.e., ranged 1.3–3.3) (Raich and Schlesinger, 1992; Tjoelker et al., 2001). The lower Q10 values (1.89–2.34) in the growing season may indicate that abiotic and biotic factors interacted to stablize ecosystem CO_2 emission.

In our study, there was no apparent acclimation of R_s under nitrogen fertilisation treatment (Table 2.4). This result is consistent with that of a short-term study in a pleioblastus amarus plantation (Tu et al., 2010), where three nitrogen addition levels have no effect on Q10. Our result also indicated that there was no apparent acclimation of R_s after warming (Table 2.4). This result accords with a meta-analysis of R_s under warming, which suggested that R_s of terrestrial ecosystems did not acclimate to climate warming (Bond–Lamberty and Thomson, 2010). Different from warming or nitrogen addition effect, combined warming and nitrogen addition promoted the acclimation of R_s to temperature (Table 2.5). Many studies have reported that global change drivers interacted with one another to affect R_s (Reynolds et al., 2015; Shen et al., 2009; Tao et al., 2013; Wan et al., 2007; Zhou et al., 2016; Zhu et al., 2015). Our results further demonstrate the notion that warming and increasing nitrogen deposition interact with each other to promote R_s to acclimate temperature.

2.4 Summary

This study investigated the effects of warming and nitrogen addition on R_s via field manipulative experiment. Our results indicate that environmental factors, such as precipitation, temperature and soil water, play critical roles in regulating R_s in the semi-arid ecosystem. We also found that nitrogen addition stimulated R_s. In addition, warming and nitrogen addition combination decreased the Q10 values of R_s but both of them separately had little impact on it, suggesting that climate warming and increasing N deposition may interactively decrease the temperature sensitivity of R_s, which may be more realistic in investigating thermal acclimation of R_s. Our results highlight the distinctive responses of R_s to warming between growing and non-growing seasons.

Chapter 3 Impacts of warming and nitrogen addition on soil autotrophic and heterotrophic respiration in a semi-arid environment

Different response mechanisms govern the behavior of of Rh and Ra to environmental drivers. For example, Ra is highly dependent on roots and associated rhizosphere, whereas Rh results from the decomposition of litter and soil organic matter by soil microbes (Hanson et al., 2000; Kuzyakov, 2002; Wan and Luo, 2003; Li et al., 2010). Other studies of the effects of soil warming have shown that Ra is mainly coupled to plant composition and the responses of productivity, photosynthesis and soil temperature (Lin et al., 2011; Li et al., 2013; Xu et al., 2015), whereas Rh is associated with the depletion of microbial biomass carbon, labile carbon, and soil temperature and water (Tucker et al., 2013; McDaniel et al., 2014; Hicks Pries et al., 2015; Chen et al., 2016b). N addition may increase soil microbial biomass and activity or decrease enzyme activity and soil organic matter (SOM) decomposability, resulting in corresponding changes of Rh (Janssens et al., 2010; Chen et al., 2017a; Wang et al., 2017). Likewise, N application may stimulate Ra due to increased plant growth or suppress Ra by reducing belowground carbon (C) allocation (Giardina et al., 2004; Zhang et al., 2014; Zhong et al., 2016b; Chen et al., 2017b; Wang et al., 2017).

To investigate the response of Rt and its components to warming and N addition, we measured Rt, Rh and Ra by using root exclusion method in a semi-arid grassland during both the growing and non-growing seasons for two years. Our objectives were to (i) examine whether the components of Rt responded differently to the concurrent increases in temperature and nitrogen loading and (ii) quantify the effects of warming and/or nitrogen addition on the relative contributions of Rh and Ra to Rt.

3.1 Materials and methods

3.1.1 Study area

The experiment was conducted from 2015 to 2016 in the Semiarid Ecosystem Research Station (Lanzhou University) on the Loess Plateau (36°02′N, 104°25′E, 2400 m above sea level). The area has a medium-temperate semi-arid climate where according to the meteorological record from 1955–2013, the mean annual air temperature is 6.5 °C and the mean growing (April to October) and non-growing (November to March) season temperatures are 11.7 °C and –5.3 °C, respectively. The mean annual precipitation was 305 mm with the 80% of the annual rainfall occurring during the growing season (April to October). The mean annual pan evaporation is roughly 1300 mm. The soil is Heima soil (Calcic Kastanozem, FAO Taxonomy) with high percentage of silt (around 76%). The study area was sown with Melilotus suaveolens L. (a biennial herb) in April 2003 to facilitate the revegetation of degraded land for improving the vegetation cover and reduce soil erosion. The plant community at the study site is dominated by Heteropappus altaicus Novopokr., Stipa breviflora Griseb., and Artemisia capillaris during the experimental period.

3.1.2 Experimental design

To evaluate the effect of warming and N addition on soil respiration, a completely random blocking design with five blocks as replicates was used in the experiment (Fig. 3.1). Four treatments in each block were included: control (Co), nitrogen addition (N), warming (W) and combined warming and nitrogen addition (WN). In each treatment, three subplots were used where the subplot area was a regular hexagon (0.5 m sides for a total of 0.65 m^{-2}). The three subplots were randomly used for Rt, Rh and aboveground biomass measurements. Microtrenching method was adopted in October 2014 for the Rh measurement of the subplots. The trenches were excavated with 0.1 m wide and 0.5 m deep and then lined with nylon mesh (0.038 mm mesh size) to prevent root growth into the plots but allow the movement of water, bacteria and soil nutrients (Zhang et al., 2014). The trenchs were then refilled with soil. The area inside the trench was then restricted from vegetation by cutting the plants regrowth manually throughout the study period.

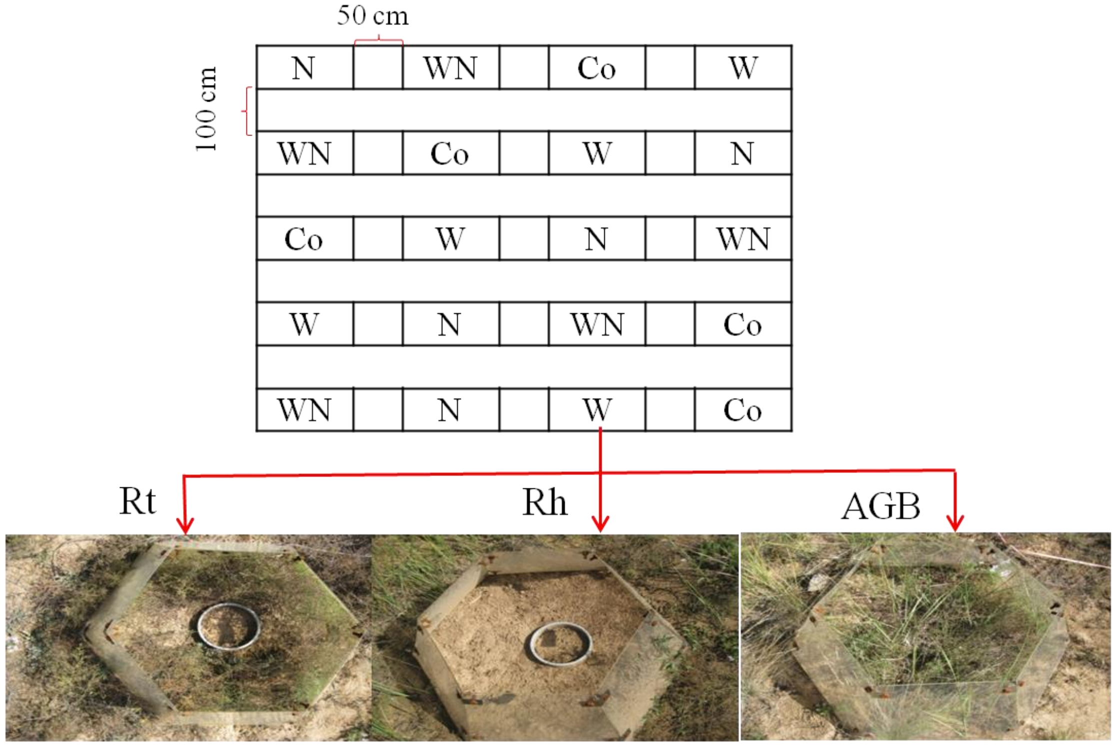


Fig. 3.1 Layout of the experiment design. WN, combined warming and nitrogen addition treatment; W, warming treatment; N, nitrogen addition treatment; Co, control treatment.

The open-top chambers (OTCs) used in the treatment plots are a hexagonal design with sloping sides of 40 cm × 50 cm × 32 cm (Maestre et al., 2013, Fang et al., 2017); the OTCs were placed on the soil surface as passive warming devices and provided warming to the enclosure throughout the entire year. The warming treatment aims to increase air temperature by 2 °C, which falls within the warming range by the year 2100 as predicted by the Intergovernmental Panel on Climate Change (IPCC 2013). Nitrogen was applied in the form of ammonium nitrate (NH_4NO_3) at a rate of 4.42 g N m^{-2} y^{-1} in the N and WN treatments starting in April 2015. This rate was chosen to represent twice the highest rate of atmospheric N deposition (approximately 2.21 g N m^{-2} y^{-1}) in the region (Lü and Tian, 2007). The total amount of nitrogen was equally divided into five applications in early April, May, June, July and August each year. In each application, NH_4NO_3 was dissolved in 65 mL distilled water and applied to each plot with a sprayer; the control plots received 65 mL distilled water. The total amount of water added to the plots by these treatments corresponded to 0.5 mm of extra precipitation annually.

3.1.3 Soil CO_2 efflux

Soil CO_2 effluxes (including Rt and Rh) were measured since April 2015. A polyvinyl chloride (PVC) collar (11 cm in diameter and 8 cm in height) was inserted in the soil with a depth of 5 cm at the center of each sampling subplot; the Li-8100 infrared gas analyzer (LI-COR Biosciences, Lincoln, Nebraska, USA) was used to measure the soil CO_2 efflux in the collar. To eliminate the effect of aboveground biomass on soil respiration (Zhou et al., 2007), living plants inside the PVC collars were clipped at the soil surface at least one day before measurements. Since litter decomposition is part of soil respiration, the clipped plants were allowed to remain inside the collars to decompose (Zhou et al., 2007; Chen et al., 2016; Jeong et al., 2016). Soil respiration was measured twice every month in the growing season and once each month in the non-growing season during the study period. All soil respiration measurements were carried out between 09:00 and 11:00 a.m. (local time). Each treatment took roughly 2–3 min. Ra was calculated as the difference between Rt and Rh.

3.1.4 Aboveground biomass

We used a non-destructive sampling method to estimate aboveground biomass (AGB) on August 13, 2016. The height of each species was measured in each plot, and AGB was calculated based on the regression equation between biomass and height for each species (Table A1).

3.1.5 Air temperature and soil temperature and moisture

Given that nitrogen addition had no significant effect on air and soil moisture and temperature (Zhang et al., 2014), we only measured these variables under warming and control treatments. One warming plot and one control plot were randomly selected for measuring air temperature, soil temperature and moisture. Air temperature was recorded by using HOBO® T/RH U23-002 data loggers (Onset Computer Corporation, Bourne, Massachusetts, USA); soil temperature and volumetric soil moisture were recorded at a depth of 5 cm using a Watchdog 1000 Series Micro station-T/RH (Spectrum Technologies Inc., Plainfield, IL, USA). All data were recorded at a 1-h interval for the two years. Although soil temperature and moisture were recorded only in one plot of each of the warming and control treatments, the values recorded were consistent with our previous study conducted in a nearby field (Fig. A1).

3.1.6 Temperature sensitivity of soil respiration

Based on the soil respiration and temperature data measured over time, the temperature sensitivity of soil respiration (Q_{10}) was determined by fitting the exponential function (see Eqn 3-1 below) for each individual treatment (Luo et al., 2001).

$$SR = ae^{bT} \qquad 3\text{-}1$$

Where SR is measured soil respiration (Rt) or its components (Rh and Ra) (μmol CO_2 m^{-2} s^{-1}), T is the soil temperature (°C) at a depth of 5 cm, a is the basal soil respiration rate when the temperature is 0 °C, and the temperature sensitivity Q_{10} is the proportional rate of increase in soil respiration when soil temperature increases by 10 °C (see Eqn 3-2).

$$Q10=e^{10b} \qquad 3\text{-}2$$

3.1.7 Statistical analysis

We performed a paired-t test to compare the difference in soil temperature and moisture for the paired warming and control treatments. Two-way analyses of variance (two-way ANOVAs) were employed to examine the effects of warming and N addition on the cumulation and Q10 of Rt, Rh and Ra. Repeated measures of ANOVAs test were adopted to examine the main and interactive effects of warming, N addition and measurement date (day) and year on Rt, Rh and Ra. Significant differences were evaluated at the level $P < 0.05$. Exponential and quadratic fittings were used to evaluate the relationships between soil respiration (Rt, Rh and Ra) and abiotic and biotic factors (soil temperature and moisture, and AGB). All statistical analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA).

3.2 Results

3.2.1 Microclimate

The annual total precipitation amount was 294 mm in 2015 and 284.8 mm in 2016, and it showed seasonal dynamics with peak values occurred on 3 August 2015 and 18 July 2016 (Fig. 3.2A). Air and soil temperature and soil moisture also displayed similar seasonal dynamics. The maximum soil temperature happened in July 2015 and August 2016 (Fig. 3.2B). The highest soil moisture occurred in 2015 and 2016 when the rainfall events happened (Fig. 3.2A and C). Warming significantly enhanced the air and soil temperatures by 1.7 °C and 2 °C, respectively ($P < 0.05$, Fig. 3.2A and B), whereas it decreased the soil moisture by 1.7% in Rt and 1.8% in Rh ($P < 0.05$, Fig. 3.2C). The trenching significantly increased the soil moisture by 1.5% under Co and by 1.6% under W ($P < 0.05$, Fig. 3.2c). No significant trenching effects on soil temperature were observed whether under Co or W ($P > 0.05$).

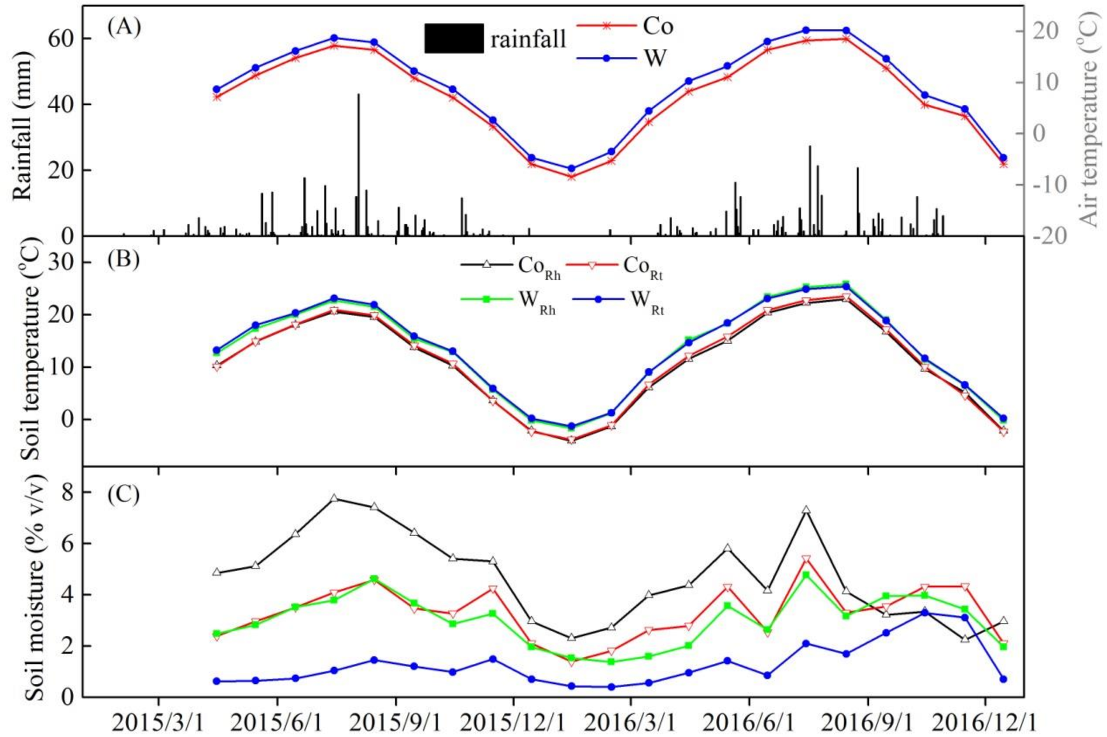


Fig. 3.2. Seasonal variations of precipitation (A), air temperature (B), and soil temperature and moisture at a soil depth of 5cm (C) from 2015 to 2016. Co, control treatment; W, warming treatment. W_{Rt}, warming treatment in Rt; Co_{Rt}, control treatment in Rt; W_{Rh}, warming treatment in Rh; Co_{Rh}, control treatment in Rh.

3.2.2 Temporal variation in soil respiration and its components

The seasonal dynamics of Rt and its components tended to follow the seasonal dynamics of precipitation and temperature (Figs. 3.2 and 3.3). The maximum values occurred in July, and the minimum values occurred in winter. Repeated measures of ANOVAs showed that measurement date interacted with concurrent warming and N addition to affect Rh, Ra and Rt, except that no interactive effects between day and combined warming and N addition in 2015 on Ra, and no interactive effects between day and warming or N addition in 2016 on Ra were observed (Table 3.1). In addition, year interacted with warming to affect Ra, but no siginificantly interactive effects between year and warming on Rh and Rt were observed. Likewise, no interactive effect was observed between year and N addition on Ra, Rh and Rt. Significant combined effect was observed between warming and N addition on Rh and Ra ($P < 0.05$, Table 3.1). Warming significantly decreased Rh by 3.9% and Rt by 2.9% in 2015, and reduced Rh by 9.48% and Rt by 7.4% in 2016 (Fig. 3.3, Table 3.1). N addition significantly increased Ra by 47.8% in 2015 and 34% in 2016 (Fig. 3.3, Table 3.1).

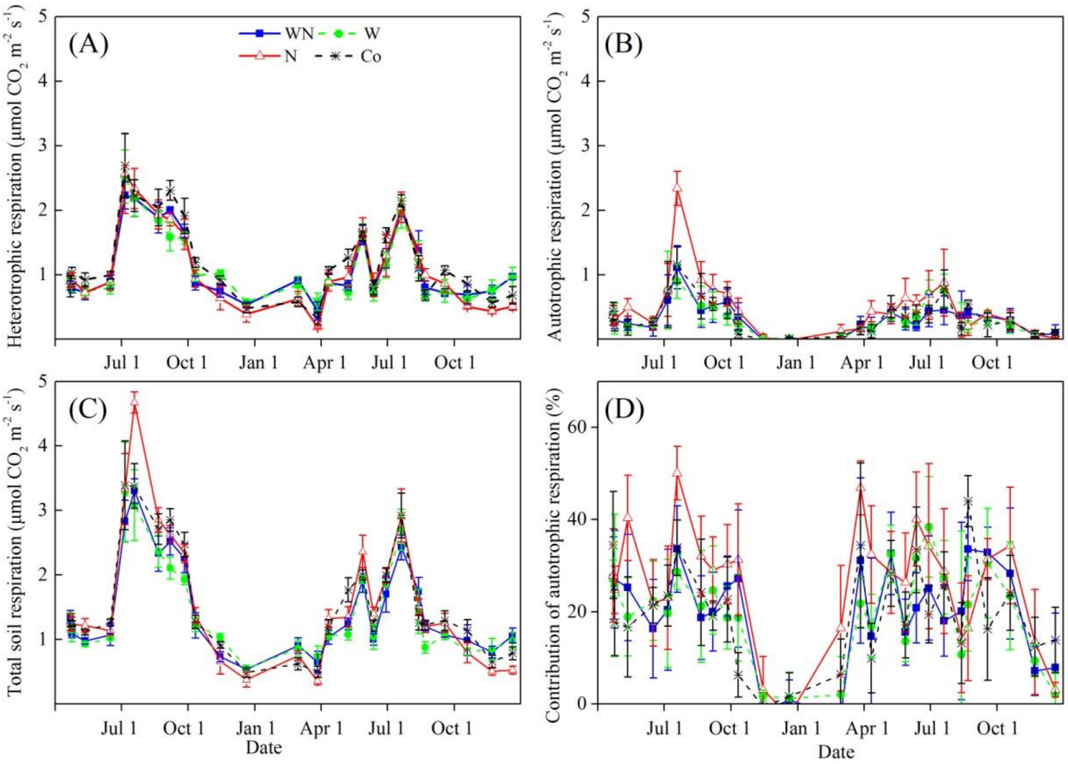


Fig. 3.3. Seasonal dynamics of heterotrophic respiration (Rh) (A), autotrophic respiration (Ra) (B), soil respiration (Rt) (C) and the contribution of Ra to Rt (d) in 2015 and 2016. WN, combined warming and nitrogen addition; W, warming; N, nitrogen addition; Co, control. Data shown are means ± standard deviation.

Table 3.1 Results of repeated measures ANOVAs of the effects of Day or Year, Warming (W), N addition (N), and their interaction on soil respiration (Rt) and its components (Rh and Ra) (μmol CO$_2$ m^{-2} s^{-1}) (n=5). The × sign suggests interaction or combined effect.

Year	Factor	Rh F-value	Ra F-value	Rt F-value
2015	Day	273.559^{***}	27.607^{***}	285.805^{***}
	Day ×W	4.209^{***}	2.905^{***}	11.157^{***}
	Day ×N	4.744^{***}	3.314^{***}	4.618^{***}
	Day ×W × N	3.146^{***}	1.427	2.456^{**}
	W	5.955^{*}	1.778	9.495^{*}
	N	4.687^{*}	5.602^{*}	0.009
	W ×N	3.713^{+}	3.185^{+}	0.409
2016	Day	206.964^{***}	13.576^{***}	153.666^{***}
	Day ×W	12.874^{***}	1.666	6.688^{***}
	Day ×N	3.368^{***}	1.018	2.284^{**}
	Day ×W × N	2.486^{**}	3.556^{***}	2.202^{*}
	W	6.376^{*}	2.622	7.991^{*}

	N	7.135^{*}	6.376^{*}	0.131
	W×N	8.629^{**}	2.406	0.620
2015-2016	Year	364.506^{***}	64.955^{***}	704.365^{***}
	Year×W	0.201	9.089^{**}	0
	Year×N	1.009	4.006^{+}	0.306
	Year×W×N	0.375	10.239^{**}	0.666
	W	0.01	16.72^{***}	19.027^{+}
	N	2.069	5.619^{*}	0
	W×N	4.779^{*}	9.932^{***}	0.109

+, *, **, and *** represents significant at $P < 0.1, 0.05, 0.01, 0.001$, respectively.

3.2.3 Cumulative soil respiration and its components

Over the two years, warming significantly decreased the cumulative Rt by 10.5% in 2015 and 9.1% in 2016, whereas N addition had no significant effect on cumulative Rt (Fig. 3.4). On the contrary, warming had no significant effect on the cumulative Ra, whereas N addition significantly stimulated the cumulative Ra by 46.8% in 2015 and 25.1% in 2016 (Fig. 3.4). Both warming and N addition significantly decreased the cumulative Rh by 11% and 12.1% in 2015 and 10.3% and 12.0% in 2016, respectively (Fig. 3.4). In addition, warming had no significant effect on the contribution of Ra to Rt but N addition significantly enhanced it by 10% in 2015 and 7% in 2016 (Fig. 3.4). Interactive effects of warming and N addition were observed on the cumulative Rh and Ra over the two years (Table 3.2).

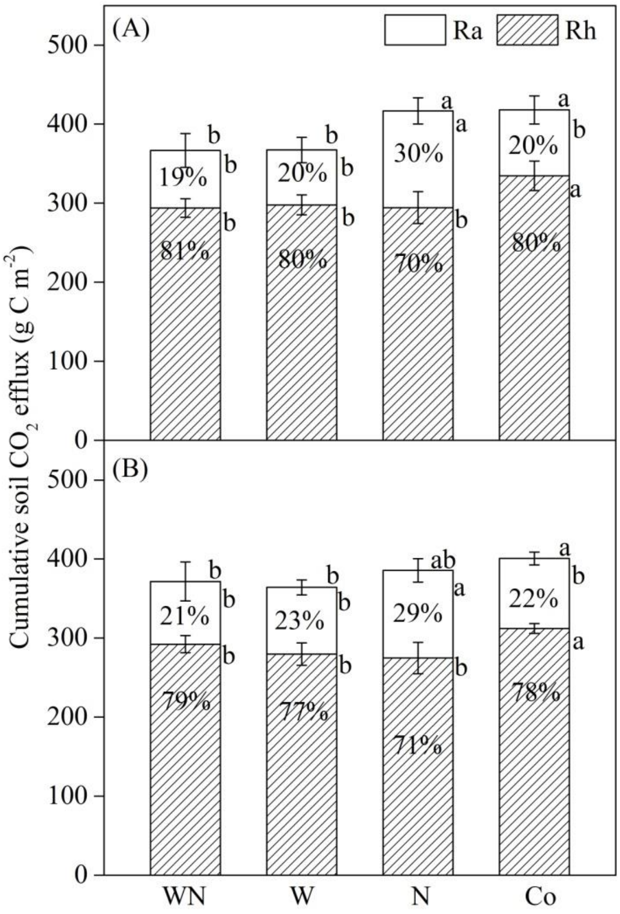


Fig. 3.4. The contribution of heterotrophic respiration (Rh) and autotrophic respiration (Ra) to soil respiration in 2015 (A) and 2016 (B) under control (Co), nitrogen addition (N), warming (W), warming with nitrogen addition (WN). Different letters represent significant difference at *P* < 0.05. Data shown are means ± standard deviation.

Table 3.2 Results (F-Values and effect size) of two-way ANOVAs of the effects of Warming (W), N addition (N), and their interaction on cumulative soil CO_2 efflux (g C m^{-2}) (n=5)

Year	Factor	Rh		Ra		Rt	
		F-value	Effect size[#]	F-value	Effect size	F-value	Effect size
2015	W	6.566^{*}	0.291	8.224^{***}	0.485	26.790^{***}	0.626
	N	9.333^{**}	0.368	5.33^{*}	0.365	0.158	0.010
	W×N	6.273^{*}	0.282	13.81^{*}	0.322	0.218	0.013
2016	W	1.456	0.083	6.568^{*}	0.291	8.951^{**}	0.359
	N	4.106^{+}	0.204	1.513	0.086	0.205	0.013
	W×N	16.517^{***}	0.508	3.805	0.192	1.773	0.100

+, *, **, and *** represents significant at *P* < 0.1, 0.05, 0.01, 0.001, respectively.

[#] Effect size is determined by partial Eta^2 provided by SPSS.

3.2.4 Interactive effect of warming and N addition

The combined effect of warming and N addition on Ra was significantly lower than the additive effect (*P* < 0.05, Fig. 3.5). However, the combined effect of warming and N addition on Rh was significantly higher than the additive effect of warming and N addition in 2015 (*P* < 0.05, Fig. 3.5A), whereas no significant difference was observed between the combined and additive effects in 2016 (*P* < 0.05, Fig. 3.5B).

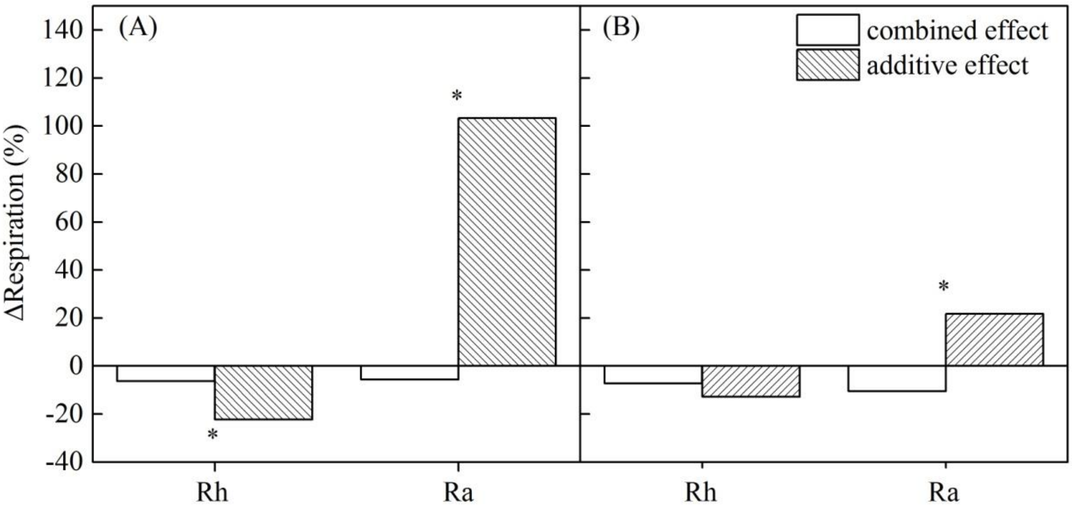


Fig. 3.5 The effect magnitude of combined warming and N addition on Rh and Ra in 2015 (A) and 2016 (B). “*” means significant different between the combined effect (WN) and the additive effect (W+N) of warming and N addition at *P* < 0.05.

3.2.5 Changes in Q10 induced by treatments

The main effects of warming and N addition on the Q10 of soil respiration and its components were significant (*P* < 0.05) except no significant main effect of warming on the Q10 of Ra in 2015 (*P* > 0.05) (Table 3.3). No significant interactive effects of warming and N addition were observed on the Q10 of soil respiration and its components (*P* > 0.05, Table 3.3). Warming significantly decreased the Q10 of Rh and Rt by 18.1% and 11.7%, respectively (*P* < 0.05), whereas it had no significant effect on that of Ra (*P* > 0.05, Fig. 3.6). N addition significantly enhanced the same Q10 of Rh and Rt by 12.8 %, but decreased the Q10 of Ra by 32.4 % (*P* < 0.05, Fig. 3.6).

Table 3.3 Results (F-Values) of two-way ANOVA on the effects of Warming (W), N addition (N), and their interaction on the temperature sensitivity (Q_{10}) of heterotrophic respiration (Rh), autotrophic respiration (Ra), and soil respiration (Rt) (n=5).

Factor	Q_{10} of Rh		Q_{10} of Ra		Q_{10} of Rt	
	F-value	*P*	F-value	*P*	F-value	*P*
W	64.687	<0.001	0.029	0.866	49.87	<0.001
N	19.246	<0.001	12.242	<0.01	16.343	<0.001
W×N	0.535	0.475	0.159	0.695	3.208	0.092

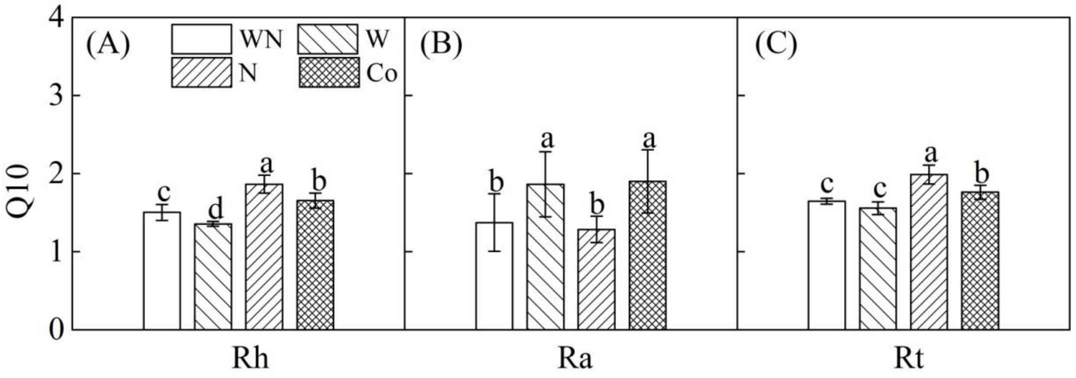


Fig. 3.6 Temperature sensitivity (Q_{10}) of heterotrophic respiration (Rh) (A), autotrophic respiration (Ra) (B), and soil total respiration (Rt) (C) during experimental period under control (Co), nitrogen addition (N), warming (W), warming and nitrogen addition (WN). Different letters represent significant difference at $P < 0.05$. Data shown are means ± standard deviation.

3.2.6 Dependence of soil respiration on biotic and abiotic factors

Rs and its components (Ra and Rh) increased exponentially with soil temperature ($P < 0.001$ for Rt and Rh, and $P < 0.05$ for Ra; Fig. 3.7A). Quadratic relationships were found between soil respiration and soil moisture ($P < 0.001$, Fig. 3.7B). Ra increased linearly with above-ground biomass ($P < 0.05$, Fig. 3.7C).

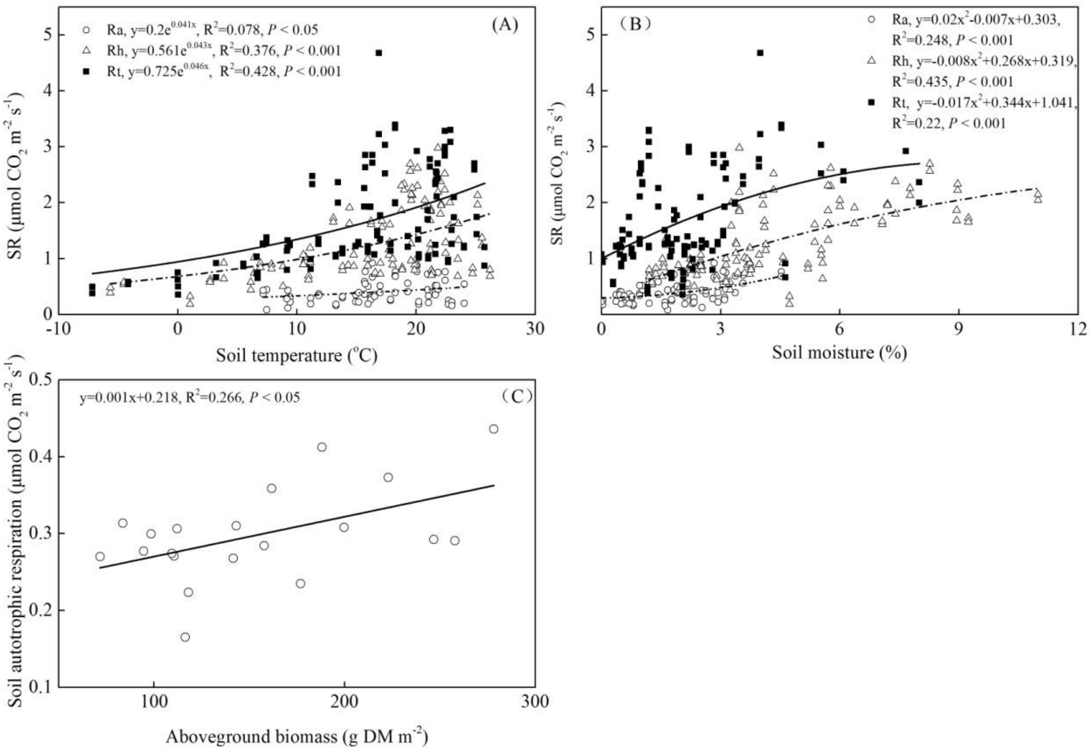


Fig. 3.7 Relationships between soil respiration (SR) (total soil respiration, Rt; soil autotrophic respiration, Ra; soil heterotrophic respiration, Rh) and soil temperature (A), soil moisture (B), and aboveground biomass (C) over the two years (2015-2016).

3.3 Discussion

3.3.1 Effects of warming on soil respiration and its components

We found that experimental warming significantly decreased Rt and Rh over the two years. Many field experiments found that warming reduced soil moisture (Wan et al., 2007; Tao et al., 2013; Wang et al., 2014; Hicks Pries et al., 2015; Reynolds et al., 2015; Fang et al., 2017). Water is the main limitation factor in the semi-arid or arid ecosystems (Ni, 2003; Miyazaki et al., 2004; Shinoda et al., 2010; Walter et al., 2011; Grossiord et al., 2017), reduced soil moisture could also decrease microbial activity (Sheik et al., 2011) and thus lead to an inhibition of Rh (Fig. 3.4). As Rt is mostly contributed by Rh (Fig. 3.4; Zhang et al. 2014), Rh reduction also led to decrease in Rt.

Ra was regulated by both aboveground biomass and soil moisture (Yan et al., 2009). In this study, Ra was significantly and linearly correlated with soil moisture and AGB (Fig. 3.7A and B). Warming resulted prior to the start of the growing season (Table A2) which might increase AGB (Fig. A2). Increased AGB might offset the negative effect of decreased soil water (Fig. 3.2) on Ra, consequently no significant change was observed in Ra. These results further emphasized that plant substrate supply and soil moisture played crucial roles in regulating Rt and its components.

3.3.2 Effects of N addition on soil respiration and its components

We found no significant response of Rt to N addition in this semi-arid grassland. Our finding is consistent with those observed in a temperate grassland (Luo et al., 2016) and an alpine meadow (Zhu et al., 2015). By contrast, several studies have shown that N addition can either increase Rt in grassland due to the positive effect on autotrophic respiration (Zhou et al., 2014), or decrease Rt due to the suppression of Rh (Yan et al., 2009). Insufficiently significant response of Rt to N addition in our study is due to the opposite responses between Ra and Rh (the effect sizes are similar for Ra and Rh), which is consistent with a meta-analysis that N addition led to an increase in Ra but an inhibition of Rh in grasslands (Zhou et al., 2014). The stimulation of Ra with N addition can be explained by the increased plant productivity (Fig. A2). Yan et al. (2009) showed that N addition enhanced Ra as a result of increased plant productivity in a temperate steppe. Ra was positively and linearly correlated with the aboveground productivity (Fig. 3.7C). Thus, a stimulation of Ra under N addition was observed. On the other hand, NH_4NO_3 is a strong acid-weak base salt that can induce soil acidification or decreased in pH. Previous studies have indicated that soil pH affects soil enzymatic activity through changing the composition and structure of soil microbial community (Craine et al., 2010; Pastorelli et al., 2013; Min et al., 2014). Hence soil acidification or decreased pH might inhibit the microbial activity, leading to an inhibition of Rh. Therefore, our results suggest that the Rt has the potential to resist N addition by adjusting the responses of its endogenous components. N addition had the highest stimulation of Ra and had no significant inhibition of Rh during the occurrence of most rain events in the month, which indicated that extreme rainfall frequency could mitigate the negative effect and promote the positive effect of N addition on soil respiration in this semi-arid environment. Chen et al. (2017b) also reported that extreme rainfall and snowfall could regulate the effect of N addition on soil respiration.

3.3.3 Interactive effect of warming and N addition on soil respiration

We found no interactive effect of warming and N addition on Rt observed (Tables 1 and 2). A similar result was observed in a Harvard Forest ecosystem, where no significant interaction was observed between warming and N fertilization on soil respiration (Contosta et al., 2011). In our study, this condition could be due to the opposite responses of Ra and Rh to the combined warming and N addition. The synergism between warming and N addition in Rh offsetted the antagonism between warming and N addition in Ra (Fig. 3.5), leading to no observed combined effect of warming and N addition on Rt. Thus, we should more consider the response of soil respiration’s inherent components to climate change for accurately modelling ecosystem carbon cycle.

3.3.4 Seasonal variation of soil respiration and Q10

Soil temperature typically exhibits seasonal variation, which mainly accounts for the temporal variations of soil respiration, especially in temperate region (Zhou et al., 2015). Ra, Rh and Rs tended to closely follow this pattern (Figs. 3.2B and 3.3).

Q10 is considered as an important parameter for assessing temperature acclimation of soil respiration (Luo et al., 2001; Luo et al., 2009; Shen et al., 2009; Xu et al., 2015). Previous studies suggested that temperature acclimation could be attributed to the depletion of labile C supply, physiological acclimation of microbes or plant and/or changes in microbial or plant community composition (Raich and Schlesinger, 1992; Luo et al., 2001; Zhou et al., 2007). In our study, the Q10 values of Rt ranged from 2.2–2.9 and were within the range (1.8–3.0) reported by a previous study in this region

(Zhang et al., 2014), and the global average value (i.e., ranged 1.3–3.3) (Raich and Schlesinger, 1992; Tjoelker et al., 2001). Our study found that warming reduced the Q10 values of Rt (Fig. 3.5C). Many previous studies have reported that warming could promote the temperature acclimation of soil respiration (Luo et al., 2001; Niinistö et al., 2004; Zhou et al., 2007; Wang et al., 2014). In fact, the reduction in Q10 might be more closely related to the response of Rh to warming. In this study, warming reduced the Q10 of Rh but did not significantly affect Ra, leading to the Q10 reduction of Rt. Grogan et al. (2005) suggested that aboveground plant biomass could regulate the temperature acclimation of soil respiration where removal of plants and litter could decrease the temperature sensitivity of soil respiration. Meanwhile, Liu et al. (2016) reported that drought stress could decrease the Q10 of soil respiration mainly due to decreased root biomass and microbial biomass carbon. In our study, warming increased the aboveground biomass and decreased the soil moisture (Figs. A2 and 3.2C). Higher aboveground biomass resulted in more litter entering into soil, which might offset the effect of drought stress on Ra and lead to no change in Q10. Whereas lower soil moisture could decrease the microbial activity and thus reduced Q10 of Rh. On the contrary, N addition increased the Q10 values of Rt and Rh whereas decreased that of Ra (Fig. 3.6). The enhancement in Rt may be attributed to the low soil temperature (annual average 10.6 °C) in our study. Zhong et al. (2016) reported that N enrichment is more likely to increase Q10 in colder or warmer region (Zhong et al., 2016a). Thus, the warming- or N addition-induced change in Q10 of Rt may be mainly regulated by Rh in the semiarid grassland.

3.3.5 Trenching effect

The micro-trenching method, which is one of the important methods for distinguishing Rh from Rt (Hanson et al., 2000; Kuzyakov, 2002), has been widely used in many ecosystems, such as grassland (Zhang et al., 2014), forest (Hanson et al., 2000; Liu et al., 2016; Zeng et al., 2016) and cropland (Moyano et al., 2007; Li et al., 2010). However, this method may cause biases in estimating Rh. First, we found that plant exclusion resulted in higher soil moisture (about 1.5%, Fig. 3.2C), which might lead to an overestimation of Rh (Yan et al., 2009). Second, trenching might cause the underestimation of Rh due to the exclusion of substrate inputs from dead roots and root exudates (Hanson et al., 2000; Ngao et al., 2007). Nevertheless, while over- or under-estimation may be inevitable, Zhang et al. (2014) suggested that the trenching method is a feasible way to partition Rh from Rt in grassland ecosystems because this approach does not destroy the soil structure. Uncertainty can also exist in estimating Ra calculated by the difference between Rt and Rh. Even so, this subtraction method has been widely and successfully used in many previous studies (Zhou et al., 2007; Li et al., 2013; Zhang et al., 2014; Peng et al., 2015; Chen et al., 2016b). Our result is also in line with a previous study in this region (Zhang et al. 2014) where Ra contributed to Rt by 20%–40%, which indicates that any biases caused by the subtraction method are probably insignificant.

3.4 Summary

Our manipulative experiment provided unique data for investigating the responses of soil respiration and its components to simultaneous increases in temperature and N loading. N addition increased the contribution of Rh to Rt, which indicated that N addition might eventually cause Rt to be dominated by Ra. The observed response trend of Rt to warming was more likely related to Rh but relied on both Rh and Ra for its response to N addition. The contrasting responses of Ra and Rh to the combined warming and N addition resulted in no significant response in Rt. Our results highlight that Rt has the potential to resist the current climate warming and increasing N deposition by adjusting the responses of its inherent components. In addition, extreme precipitation frequency and soil moisture play crucial roles in regulating Rt and its components. This study is important for the revegetation of degraded land in this region under changing climate and nitrogen loading.

Chapter 4 Unaltered soil microbial community composition, but decreased metabolic activity in a semi-arid grassland after two years of passive experimental warming

Profiles of phospholipids fatty acids (PLFAs) have been widely used to investigate microbial biomass and community composition because PLFAs only remain intact in active or dormant cells and hence are characteristic biomarkers for living microorganisms (Evershed et al., 2006; Feng & Simpson, 2009; Frostegård & Bååth, 1996; Wei et al., 2014; Xu et al., 2015). Previous studies have shown that warming responses of total soil microbial community size (i.e. total PLFAs) are highly variable and complex. For example, warming was found to have a negative (Ali et al., 2018; Xue et al., 2016), no (Schindlbacher et al., 2011), or even a positive effect (Wang et al., 2017; B. Zhang et al., 2015) on total PLFAs. These different warming responses of total soil microbial community size may result directly from shifts of microbial functional groups, or indirectly from changes of soil and plant properties. For example, the warming treatment directly led to rapid shifts in the structure of the soil microbial community with significantly increased abundance of actinomycetes biomarkers and decreased abundance of fungi (Xiong et al., 2016). Warming may alter soil pH, soil resource availability, plant community composition, and plant production (Bai et al., 2019; Fang et al., 2018; Guan et al., 2018; Li et al., 2017; Xu, Sherry, Niu et al., 2013; Zi et al., 2018), which can all affect the soil microbial community (Feng & Simpson, 2009; C. Wang et al., 2017; Xiong et al., 2016; Xue et al., 2016). However, the mechanisms underlying warming responses of the microbial community and its different functional groups still remain unclear (Li et al., 2017; Pendall, 2018).

The overall effect of warming on soil C pool can be inferred by assessing soil respiration rate (Liski et al., 1999; Luo et al., 2009; Walker et al., 2018) which is crucially affected by microbial metabolic rate (i.e. microbial respiration rate) (Fang et al., 2018; Hicks Pries et al., 2015; Kuzyakov, 2002; Zhou et al., 2007). Studies over the past decades have shown that the warming response of microbial respiration is highly variable. Previous studies showed that warming could stimulate microbial respiration due to increased microbial biomass C (Liu et al., 2019), soil nutrient availability (Ali et al., 2018), root exudates (Li et al., 2013), enzymatic activity (Bragazza et al., 2012; Li et al., 2017), or plant production (Euskirchen et al., 2009). Some other studies showed opposite results, i.e. microbial respiration inhibited by warming, which resulted from decreased soil water availability (Fang et al., 2018; Liu et al., 2009), labile C (Li et al., 2019), microbial biomass C (Chen et al., 2016) or enzymatic activity (Garcia-Palacios et al., 2018). As a result, these contrasting responses of microbial metabolic rate to warming led to large uncertainties and contradictory predictions of climate-C feedbacks (Bradford et al., 2010; Hartley et al., 2007; Hartley et al., 2009). Thus, it is crucial to clarify the warming responses of microbial metabolic rate in terrestrial ecosystems, especially in semi-arid ecosystems, where low soil water availability is more likely to induce unexpected warming responses (Song et al., 2019).

The Loess Plateau of Northwestern China is one of the most eroded regions in the world (Chen et al., 2007; Turner et al., 2011), and, because of its size, plays an important role in regulating global C cycle and climate change (Shi et al., 2011; Ueyama et al., 2009). The semi-arid area, accounting for 60% of the Loess Plateau, is characterised by low precipitation, low soil water content and low vegetation cover, as well as severe soil erosion (Chen et al., 2007; Gao et al., 2009; Ye et al., 2013; Zhang et al., 1989). Consequently, it is extremely vulnerable to climate change. Previous studies have shown that warming may decrease soil water availability and plant cover in semi-arid grasslands of the Loess Plateau (Fang et al., 2018; Fang et al., 2017), and potentially decrease microbial metabolic rate and change microbial community composition. To evaluate the effects of warming on soil microbial dynamics, we conducted a manipulative experiment in a typical semi-arid grassland on the Loess Plateau, China. We hypothesized that the changes in soil environmental characteristics due to warming will (1) decrease microbial metabolic rate and soil microbial biomass; and (2) change soil microbial community composition.

4.1 Materials and methods

4.1.1 Study area

The experiment was conducted from April 2015 to December 2017 in a fenced grassland at the Semiarid Ecosystem Research Station (Lanzhou University) on the Loess Plateau of China (36°02′N, 104°25′E). The area has a medium-temperate semi-arid climate. The altitude of the site is 2400 m above sea level. According to the meteorological record from 1955–2013, mean annual air temperature is 6.5 °C, and mean annual precipitation is 305 mm, with 80% of the annual rainfall occurring during the growing season (April to October). The mean annual pan evaporation is roughly 1300 mm. The soil is clarified as Heima soil (Calcic Kastanozem, FAO Taxonomy), with a high percentage of silt (around 76%). The study area was sown with *Melilotus suaveolens L.* (a biennial herb) in April 2003 to facilitate the revegetation of degraded land for improving the vegetation cover and reduce soil erosion. Then the *Melilotus suaveolens L.* grassland was fenced and unmanaged. The main vegetation type at the study site was dominated by *Heteropappus altaicus Novopokr.*, *Stipa breviflora Griseb.*, and *Artemisia capillaris* through secondary succession.

4.1.2 Experiment design

Two treatments in this study were included: control and warming (Fig. 4.1). The open top chambers (OTCs) were used as passive warming devices, which were shown to be effective in a large number of experiments. The OTCs used in the present study have been described in detail in former publications (Fang et al., 2018; Fang et al., 2017). For each treatment, three randomly chosen subplots with a regular hexgon (0.5 m sides for a total of 0.65 m^{-2}) were selected for soil sampling, microbial respiration and aboveground biomass measurements, respectively. Micro-trenching method was adopted in October 2014 for soil microbial respiration measurement within each subplot (see below). The trenches (0.1 m wide and 0.5 m deep) were excavated and then lined with nylon mesh (0.038 mm mesh size) to prevent root growth into the trenched area, yet allowing the movement of water and soil nutrients (Zhang et al., 2014). The trenches were then refilled with the same soil. The area inside the trench was then kept vegetation-free by cutting those regrowing plant manually throughout the study period. Effects of the trenching method on soil properties in the same field were disccused in a fomer study (Fang et al., 2018).

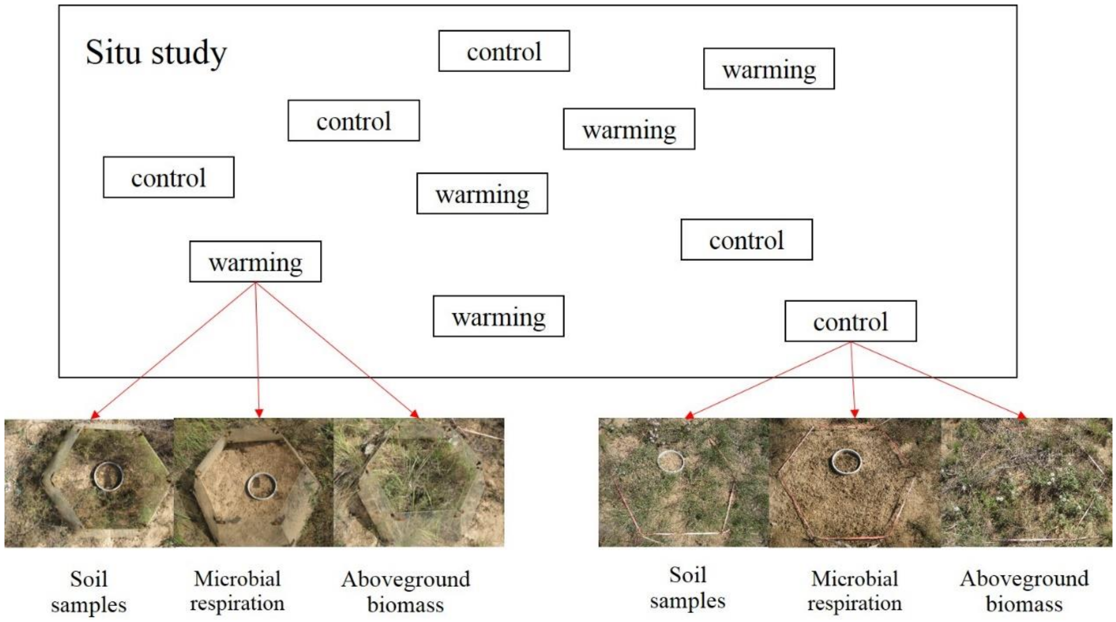


Fig. 4.1 Layout of the experiment design. In each plot, three subplots were set to measure soil properties, microbial respiration and aboveground biomass, respectively.

4.1.3 Soil sampling

In August 2017, soil samples were collected using a soil auger with an inner diameter of 2 cm. Five soil cores in each plot were taken to a depth of 20 cm and mixed into one soil sample. Soil samples were placed on ice and immediately transported to the lab. Afterwards, each soil sample was immofeuised and divided into three parts. One part was stored at 4 °C for soil moisture, microbial biomass (C and N) and inorganic nitrogen (N) measurements. Another part was stored at -20 °C for PLFAs measurement. The third part was air dried for soil pH, organic carbon (SOC), light (LOC) and heavy (HOC) fractions of SOC, total N (TN) and phosphorus (P) measurements.

4.1.4 Soil physicochemical properties and PLFAs

Soil water content was determined gravimetrically after oven-dring at 105 °C. Soil pH was measured in a soil-water solution ratio of 1:2.5 (w/w) using a glass electrode. Soil total organic carbon was measured using the Walkley and Black method (Nelson & Sommers, 1982). The density fractions of light and heavy fractions of soil organic carbon were first extracted using the method of (Gregorich & Ellert, 1993), and then determined by the Walkley and Black method (Nelson & Sommers, 1982). Soil total N was measured using the Kjeldahl digestion method (Bremner & Mulvaney, 1982). Soil total phosphorus was determined by the molybdate colorimetric method (O'Halloran &

Cade-Menun, 2006). Soil available P was determined with the Olsen method (Olsen, Cole, Watanabe, & Dean, 1954). Soil inorganic N was determined by San^{++} Automated Wet Chemistry Analyzer (Skalar, Breda, Netherlands) after extraction with KCl (2 mol L^{-1}) (Miller & Keeney, 1982). Soil microbial biomass C and N were determined by chloroform-fumigation-extraction method (Brooks, Landman, Pruden, & Jenkinson, 1985; Vance, Brookes, & D.S., 1987).

PLFAs were extracted and quantified using a modified method by Bossio and Scow (1998). Briefly, water content of the soil samples were measured prior to the procedure for adjusting the method and for further calculations. Lipids were extracted with a chloroform: methanol: phosphate buffer mixture (1:2:0.8 v/v/v, pH 4.0) from 8 g frozen soil. Throughout the procedure, teflon tubes and caps were hexane rinsed, and all glassware was heated to 121 ℃ for 3 h to sterilize and remove exogenous lipids. The phospholipids were separated from neutral lipids and glycolipids on a silica acid column (0.5 g Si, Supelco, Inc., Bellefonte, Penn). Polar lipids were eluted and then subjected to a mild alkaline methanolysis after the addition of an internal standard—methyl nonadecanoate fatty acid (19:0). The resulting fatty acid methyl esters (FAMEs) were separated, quantified and identified with an Agilent 6890 gas chromatographer (GC; Agilent Technologies, Palo Aito, CA, USA) equipped with a 19091B-102 flame ionization detector (Agilent Technologies). Samples were injected in split-less mode (injector temperature: 230 ℃) and separated using a DB23 column (60 m × 0.25 mm × 0.25 μm; Agilent, Vienna, Austria) with 1.5 ml/min Helium as the carrier gas. GC operating conditions were as follows: 1.5 min at 70 ℃, 30 ℃ min^{-1} to 150 ℃, 1 min at 150 ℃, 4 ℃ min^{-1} to 230 ℃, 15 min at 230 ℃), N_2 as the make-up gas, and air to support the flame. The fatty acid methyl esters of these samples were identified based on chromatographic retention time according to the MIDI Sherlock Microbial Identification System in the standard EUKARY chromatographic program (Microbial ID, Inc., Newark, DE, USA). The concentrations of PLFAs were standardized by the reference concentrations of internal standard (19:0) at a retention time of 71.14 min.

The terminal-branched saturated PLFAs a13:0, i13:0, a15:0, i15:0, a16:0, i16:0, a17:0, and i17:0 were used as markers for gram-positive bacteria. The mono-unsaturated and cyclopropyl saturated PLFAs 2OH 16:1, 16:1ω7c, cy17:0, and cy19:0 were used as markers for gram-negative bacteria. The bacterial markers were 12:0, 14:0, 16:0, 17:0, 18:0, G+ and G-. The 18:2ω6, 9c was used as a fungal PLFA marker. The methylic, mid-chainbranched, saturated PLFA peaks 10 Me 16:0, 10 Me 17:0, and 10

Me 18:0 were used as indicators for actinomycetes. The PLFAs 16:1ω5c and 16:1ω11 representated arbuscular mycorrhizal fungi. Total PLFAs concentration was used as an index of the total microbial biomass. The sum of PLFAs characteristic of total bacteria (Tbacteria), Gram positive bacteria (G+), Gram negative bacteria (G-), actinomycetes (ACT), fungi (F), and arbuscular mycorrhizal fungi (AMF) was used to determine broad taxonomic microbial groupings. We characterized microbial C/N, F/B, G+/G- and ratio of the sum of cyclopropyl PLFAs to the sum of their monoenoic precursors (cy 17:0+cy19:0)/(16:1ω7+18:1ω7) (CM) as the indicators of physiological or nutritional stress in microbial communities (Bossio & Scow, 1998; Fanin et al., 2019; Moore-Kucera & Dick, 2008; Schindlbacher et al., 2011).

4.1.5 Microbial respiration

Soil microbial respiration was measured from April 2015 until December 2017. A polyvinyl chloride (PVC) collar (11 cm in diameter and 8 cm in height) was inserted into the soil to a depth of 5 cm at the center of each trenched area and a Li-8100 infrared gas analyzer (LI-COR Biosciences, Lincoln, Nebraska, USA) was used to measure the soil CO_2 efflux in the collar. Microbial respiration was measured twice every month in the growing season (April to October) and once per month outside of growing season. All microbial respiration measurements (2 treatments with 5 replicates) were carried out between 09:00 and 11:00 a.m. (local time). Each measurement took roughly 2–3 min.

4.1.6 Aboveground biomass

See 3.2.4.

4.1.7 Air temperature, soil temperature and moisture

See 3.2.5.

4.1.8 Statistical analysis

We performed a paired-t test to compare the difference in soil temperature and moisture for the paired warming and control treatments. Independent-samples t test was used to verify the effect of soil warming on SWC, pH, SOC, TN, TP, AP, C/N, inorganic N, LOC, HOC, aboveground biomass, PLFAs, G+/G-, F/B, MC, microbial respiration, MBC, MBN and MBC/MBN. Significant differences were evaluated at the level $P <$ 0.05. Pearson's correlation analysis was used to evaluate the correlations among soil microbial community, indicators of environment stress, AGB, pH and soil nutrients.

Structural equation modeling (SEM) was performed using AMOS 21.0 to quantify the relative importance of the potential direct and indirect pathways in mediating the soil warming effects on soil microbial respiration based on conceptual modeling (Fig. 4.2), significance of the regression equation (Pearson's correlation analysis, Table 4.1), the goodness of model fit and logical reasoning. As the soil samples were taken in August 2017, mean value of soil microbial respiration from June to September in 2017 was used in the SEM for more accurately evaluating the driving mechanisms of warming on microbial respiration. All statistical analyses were performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

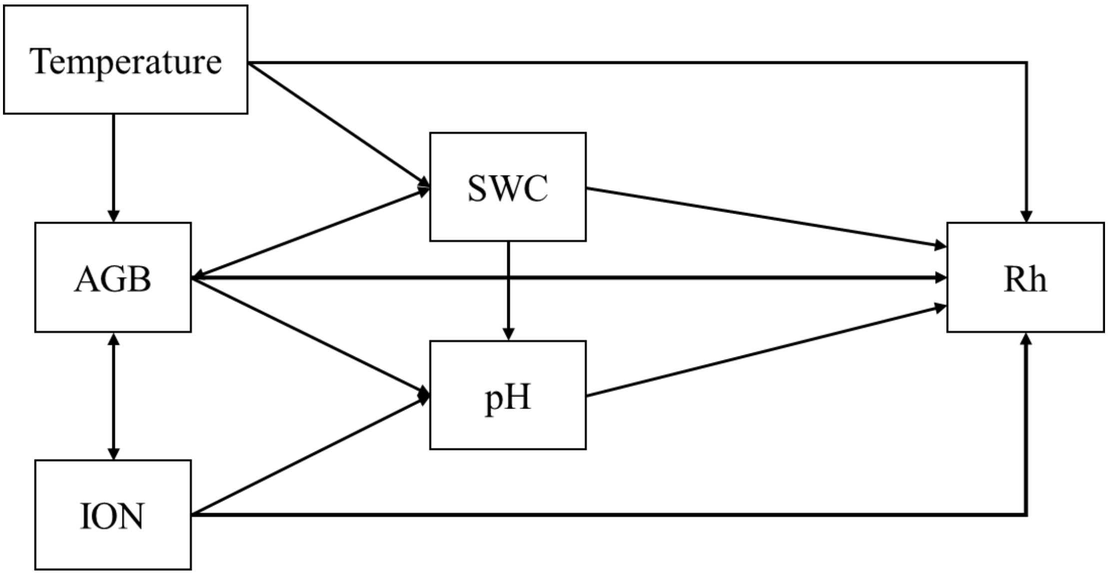


Fig. 4.2 Conceptual structure equation modelling (SEM) with variables (boxes) and potential causal relationships (arrows) for soil microbial respiration. Rh: Microbial respiration; SWC: soil water content; pH: soil pH value; AGB: aboveground biomass; ION: inorganic nitrogen.

4.2 Results

4.2.1 Soil water and chemical properties

Warming significantly decreased SWC from 8.38±0.43% to 6.05±1.18% (Fig. 4.2A). Soil pH was significantly lower in the warmed treatment than in the control treatment (Fig. 4.2B). Inorganic N and AGB were significantly higher in the warmed treatment than in the control treatment (Fig. 4.2H and K). There were no significant differences in SOC, TN, TP, AP, C/N, LOC and HOC between the warmed and control treatments (Fig. 4.2C-G and I-J).

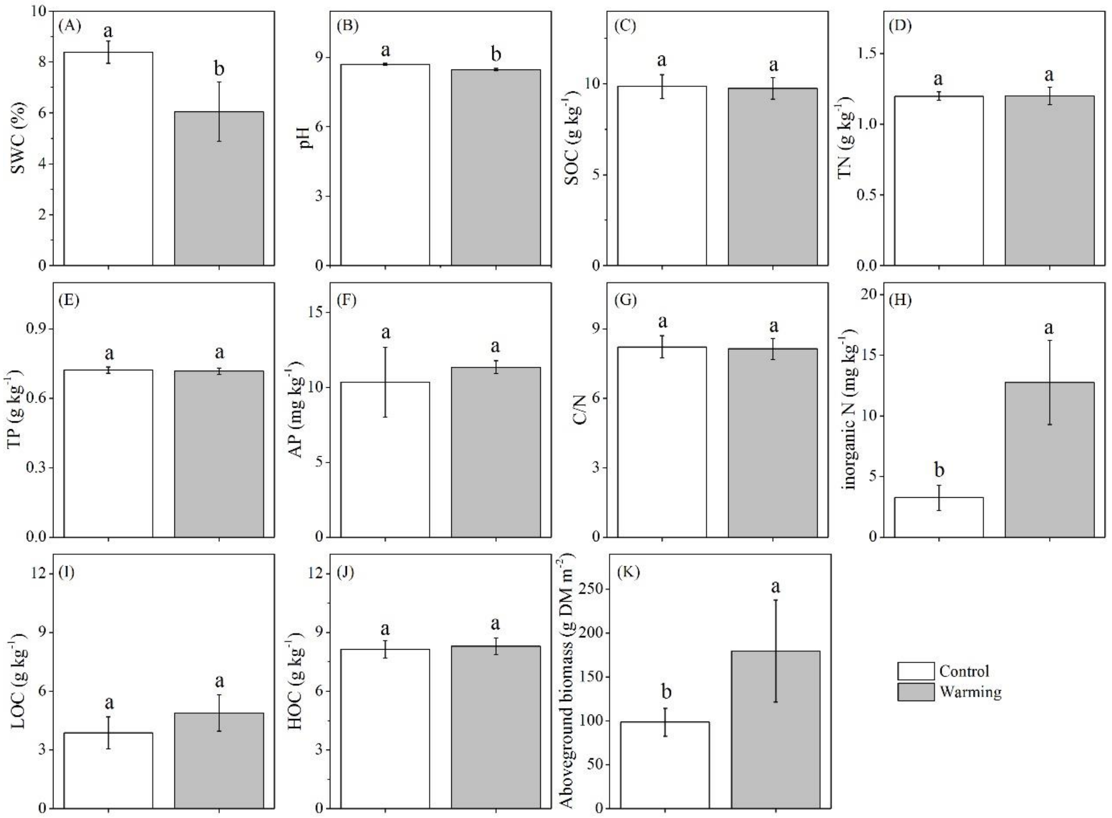


Fig. 4.3 Soil chemical characteristics at a soil depth of 20 cm. SWC: soil gravitational water content (A); pH: soil pH (B); SOC: soil organic carbon (C); TN: soil total nitrogen (D); TP: soil total phosphorus (E); AP: soil availability phosphorus (F); C/N: soil organic carbon/total nitrogen (G); inorganic N: inorganic nitrogen (H); LOC: light organic carbon (I); HOC: heavy organic carbon (J); Aboveground biomass (K). Different letters represent significant difference at $P < 0.05$. Data shown are means ± standard deviation.

4.2.2 Soil microbial properties

The PFLAs of bacteria and fungi accounted for 67.4 % and 15.4 % of TPFLAs in the control treatment, respectively. There were no significant differences in TPFLAs, actinomycetes, Tbacteria, G+, G-, fungi, AMF, G+/G-, F/B and CM between the warmed and control treatments (Fig. 4.3). Microbial respiration was significantly lower in the warmed treatment than in the control treatment (Fig. 4.4A and B). No significant differences in MBC, MBN and MBC/MBN were observed between warming and control treatments (Fig. 4.4C-E).

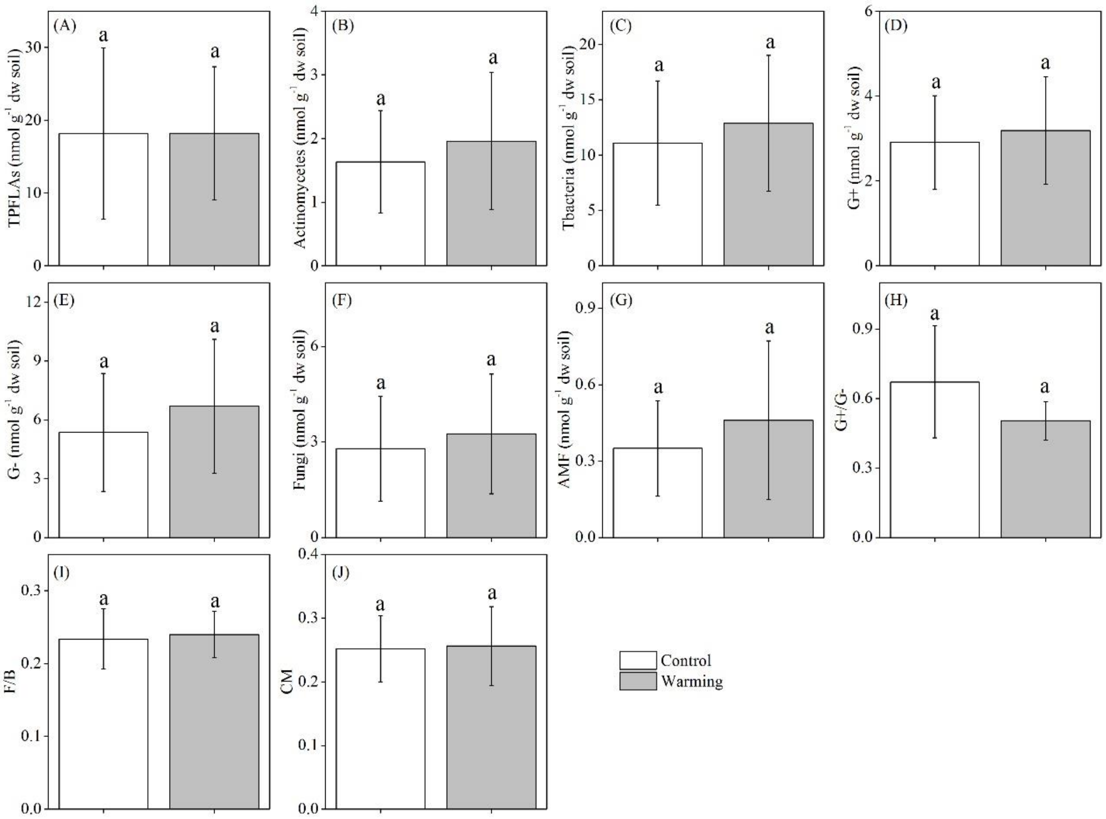


Fig. 4.4 PLFAs of total microbial community (A), Actinomycetes (B), Tbacteria (C), G+ (D), G- (E), Fungi (F), AMF (G), and G+/G- (H), F/B (I) and CM (J). TPLFAs: total PLFAs; Tbacteria: total bacteria; G+: gram-positive bacteria; G-: gram-negative bacteria; AMF: arbuscular mycorrhizal fungi; F: fungi; B: bacteria; CM: (cy 17:0+cy19:0)/(16:1ω7+18:1ω7). Different letters represent significant difference at P < 0.05. Data shown are means ± standard deviation.

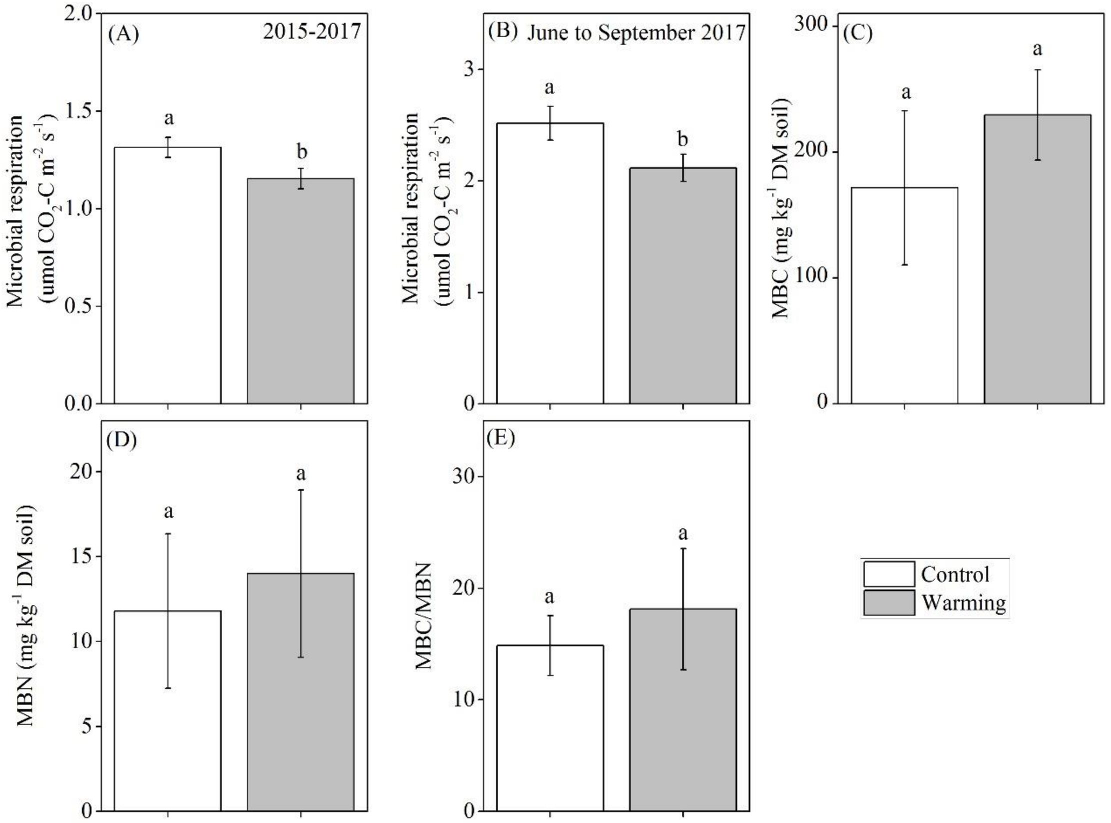


Fig. 4.5 Soil microbial respiration rate and biomass. Mean value of microbial respiration during April 2015 to December 2017 (A), Mean value of microbial respiration during June to September 2017 (B), soil microbial biomass C (MBC) (C), N (MBN) (D) and MBC/MBN (E) at the 0-20 cm soil depth. Different letters represent significant difference at $P < 0.05$. Data shown are means ± standard deviation.

4.2.3 Relationships of soil microbial properties with soil chemical properties

TFLAs, actinomycetes, Tbacteria, G+, G-, fungi, AMF were significantly correlated with TP, G+/G-, F/B and CM, except for the absence of a relationship between actinomycetes and TP (Table 4.1). Significant relationships were observed between G+/G- and F/B, G+/G and CM on the one hand, F/B and CM on the other hand (Table 4.1). Microbial respiration was significantly positively correlated with pH and SWC, and negatively correlated with inorganic N (Table 4.1). The final SEM of microbial respiration showed that AGB, inorganic N, SWC and pH explained 63% of the variance in microbial respiration (Fig. 4.5). Taking the direct and indirect effects together, inorganic N was the most important predictor shaping the variance of microbial respiration (Fig. 4.5). Specifically, AGB, inorganic N and SWC affected microbial respiration through directly regulating pH (Fig. 4.5).

Table 4.1 Pearson correlation coefficients (r values) between soil microbial community and soil nutrients and indicators of environment stress. TPLFAs: total PLFAs; Tbacteria: total bacteria; G+: gram-positive bacteria; G-: gram-negative bacteria; AMF: arbuscular mycorrhizal fungi; MBC: soil microbial biomass C; MBN: soil microbial biomass N; Rh: microbial respiration; C/N: soil organic carbon/total nitrogen; MBC/MBN: soil microbial biomass C/soil microbial biomass N; F/B: fungi: bacteria; CM: (cy 17:0+cy19:0)/(16:1ω7+18:1ω7); AGB: aboveground biomass; pH: soil pH; SWC: soil gravitational water content; ION: inorganic nitrogen; SOC: soil organic carbon; LOC: light organic carbon; HOC: heavy organic carbon; TN: soil total nitrogen; TP: soil total phosphorus; AP: soil availability phosphorus.

Microbes	AGB	pH	SWC	ION	SOC	LOC	HOC	TN	TP	AP	C/N	MBC/MBN	G+/G-	F/B	CM
TPLFAs	0.36	0.17	0.25	0.11	0.43	0.08	-0.43	0.03	-0.66*	-0.29	0.46	0.06	-0.65*	0.84**	-0.72*
Actinomycetes	0.57	0.09	0.02	0.16	0.38	0.23	-0.28	0.07	-0.47	-0.12	0.37	-0.01	-0.67*	0.80**	-0.59*
Tbacteria	0.54	0.06	0.12	0.23	0.32	0.26	-0.38	0.11	-0.63*	-0.23	0.28	-0.03	-0.75*	0.88**	-0.68
G+	0.55	0.10	0.15	0.20	0.40	0.21	-0.40	0.09	-0.60*	-0.20	0.39	-0.01	-0.67*	0.84**	-0.68*
G-	0.56	0.01	0.09	0.29	0.27	0.32	-0.38	0.14	-0.65*	-0.20	0.21	-0.02	-0.78**	0.89**	-0.68*
Fungi	0.52	0.06	0.16	0.24	0.27	0.29	-0.41	0.10	-0.66*	-0.24	0.24	-0.12	-0.74*	0.91**	-0.73*
AMF	0.55	-0.07	0.21	0.40	0.26	0.43	-0.44	0.26	-0.73*	-0.16	0.12	0.05	-0.67*	0.85**	-0.75*
MBC	0.56	-0.29	-0.42	0.34	0.13	-0.13	-0.10	-0.17	-0.34	-0.20	0.28	-0.55	-0.75*	0.43	-0.01
MBN	0.48	-0.14	0	0.25	-0.04	-0.17	-0.30	0	-0.37	-0.22	-0.02	-0.33	-0.52	0.37	-0.03
Rh	-0.56	0.79**	0.75**	-0.74*	0.21	-0.24	-0.06	0.16	0.02	-0.47	-0.11	-0.15	0.13	0.22	-0.36
C/N	0.29	0.28	-0.07	-0.15	0.79	-0.16	-0.01	-0.20	0.04	-0.21	1.00	0.28	0.04	0.02	0.05
MBC/MBN	-0.02	-0.28	-0.62	0.15	0.03	0.21	0.29	-0.37	0.05	0.04	0.28	1.00	-0.19	0.01	-0.01
G+/G-	-0.42	0.24	0.23	-0.38	0.07	-0.2	0.25	0.05	0.65*	0.27	0.04	-0.19	1.00	-0.8	0.44
F/B	0.32	0.02	0.2	0.23	-0.10	0.16	-0.62	-0.16	-0.82*	-0.20	0.02	0.01	-0.80**	1.00	-0.84**
CM	-0.10	0.03	-0.41	-0.26	0.12	-0.23	0.65*	0.11	0.82*	0.10	0.05	-0.01	0.44	-0.84**	1.00

*, $P<0.05$, **$P<0.01$

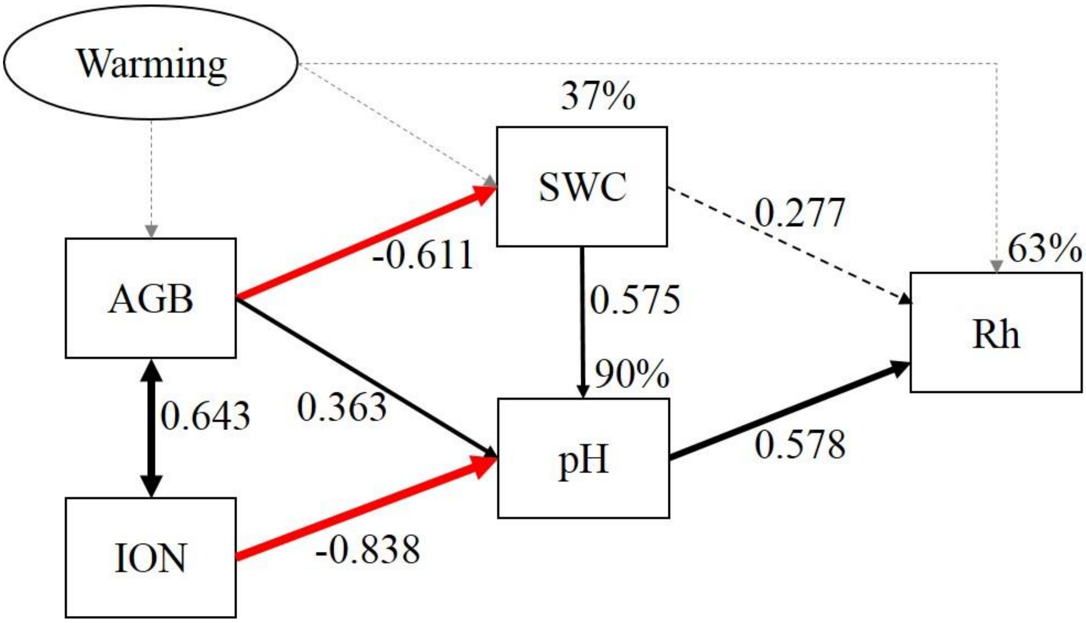


Fig. 4.6 Structure equation modelling (SEM) with variables (boxes) and potential causal relationships (arrows) for soil microbial respiration ($\chi^2 = 5.154$, df = 3, P = 0.161 > 0.05, CFI = 0.937 > 0.9). Rh: Microbial respiration; SWC: soil water content; pH: soil pH value; AGB: aboveground biomass; ION: inorganic nitrogen. Single headed arrows represent the hypothesized direction of causation. Numbers next to single headed arrows are standardized path coefficients, which indicate the effect size of the relationship. The double headed arrow represents the covariance between related variables. Red and black arrows indicate negative and positive relationship. The proportion of variance explained appears above each response variables in the model. Solid line means significance and dash line means non-significance. Due to insufficient temperature data (only continuous measurements in two plots in two treatments) for a SEM analysis, the grey dashed lines represent the conceptual warming effect.

4.3 Discussion

Our hypothesis that increased temperature would decrease soil microbial biomass and alter community composition was not supported, results showed that two years of warming did not affect soil microbial biomass and community composition. This finding is consistent with those observed in the forest soils with soil warming of 4-5 ^{o}C (Contosta et al., 2011) and an alpine meadow with soil warming of 0.44 ^{o}C (Zi et al., 2018).

The mechanism underlying the lack of a warming response in microbial biomass and community composition might result from the abiotic and biotic factors coupled in this study. In our study, warming significantly increased soil temperature and stimulated aboveground biomass, which resulted in increased litter production and this accelerated soil N cycling (e.g. increased N availability) (Fig. 4.2), providing a favorable environment for the soil microbial community. In a lab incubation study, increased temperature decreased total PLFAs due to decreased substrate availability (Ali et al., 2018). In an other laboratory soil warming study, Zhou et al. (2017) reported that increased total PLFAs at higher temperatures was attributed to stimulated substrate availability, providing a favorable environment for soil microbes (Zhou et al., 2017). However, decreased soil moisture and pH provided unfavorable elements for the soil microbial community (Fig. 4.2). In an alpine meadow, warming altered microbial community composition due to decreased pH and vegetation coverage causing an unfavorable environment for microbial community (Yu et al., 2019). Consequently, these antagonistic abiotic and biotic factors may have offset each other, resulting in the absence of a warming effect on soil microbial biomass and community composition (Figs. 4.3 and 4.4). These results suggest a resistance of the soil microbial community to short-term warming in the studied semi-arid grassland ecosystem.

A review study on terrestrial ecosystems reported that a shift in soil microbial community composition occurred after an average of three years warming (Allison & Martiny, 2008). In some cases, even more than a decade may be required to detect significant warming responses of the microbial community composition (Rinnan et al., 2007). However, Dajana et al (2017) reported that long-term (> 50 years) and even long-term (5-7 years) soil warming did not induce the shifts of soil microbial community composition but significant shifts for bacteria and fungi were observed starting from + 9 °C in the long term and +7 °C/+3 °C in the short term in the sub-arctic grasslands. Thus, long-term or higher temperature increase experiments are also necessary to detect if there are significant responses of soil microbial biomass and community composition to warming in grasslands. Such long-term experiments are planned at the site..

Our hypothesis that warming would decrease microbial respiration was confirmed. This result is consistent with studies in the semi-arid alpine steppes (Liu et al., 2009; Zhao et al., 2019; Zhou et al., 2013). Previous studies have shown that the warming response of microbial respiration depends on various abiotic and biotic determinants, such as substrate N availability, pH, soil temperature and moisture, and plant production (Bradford et al., 2010; Chang et al., 2014; Hicks Pries et al., 2015; Li et al., 2013; Moyano et al., 2007; Peng et al., 2015; Zhou et al., 2007). Wan et al (2005) reported that warming increased aboveground biomass, subsequently causing soil available N increasing in a tallgrass prairie (Wan et al., 2005), which had a positive effect on soil microbial respiration (Hadas et al., 2004). However, too high inorganic N may induce N toxicity (decreased osmotic potential or pH), which can inhibit soil microbial respiration (Treseder, 2008). The northwest area of China received a high N deposition rate with 2.21 g m^{-2} yr^{-1} (Liu et al., 2011), where soil microbes may experience N toxicity in a very high inorganic soil induced by warming (Fig. 4.2H). Zeng et al (2018) also reported that the potential positive effect of soil inorganic N on soil microbial respiration was suppressed by the negative effect from decreased pH in temperate arid grasslands.

In addition, in warming treatments, the positive effect of increased temperature on microbial respiration could be suppressed by decreased soil water content or water stress induced by warming (Chang et al., 2014; Liu et al., 2016). Many field experiments found that warming reduced soil moisture (Hicks Pries et al., 2015; Tao et al., 2013; Wan et al., 2007; X. Wang et al., 2014). Water is the main limiting factor in semi-arid or arid ecosystems (Walter et al., 2011), and reduced soil moisture can decrease microbial metabolic rate (Liu et al., 2009). A former study in the same site showed that around 2 year warming (April 2015 to December 2016) decreased microbial respiration but the over all driving mechanisms remained unclear (Fang et al., 2018). To evaluate whether the reduced soil water content or the other soil factors underlay the warming response of microbial respiration, SEM was used to provide a better and more systematic understanding.

The results of SEM suggested that the decreased soil moisture and pH, and increased AGB and inorganic N co-determined the negative warming response of soil microbial respiration (Fig. 4.5). The decline in soil microbial respiration following warming was directly linked to changes in soil pH, and indirectly linked to changes in AGB, inorganic N and soil moisture. On the one hand, increased AGB decreased soil moisture, which subsequently had a positive effect on pH, causing a negative effect on soil microbial respiration. On the other hand, negative effect of inorganic N on pH overrode the positive effect of AGB inducing an observably decreased pH, causing a negative warming effect on soil microbial respiration. These driving pathways demonstrated that warming responses of microbial respiration resulted from a combination of multi-driving paths from abiotic and biotic factors.

4.4 Summary

We observed that warming did not change soil microbial community composition and biomass but decreased soil microbial respiration. The lack of warming responses of soil microbial community composition and biomass were due to their resistance to the short term and limited (2 ^{o}C) warming. Decreased soil microbial respiration was attributed to the co-mediation of soil temperature, moisture, inorganic N, pH and aboveground biomass. These findings further revealed that, in the short term the soil microbial community can resist to a warmer climate by decreasing their metabolic rate in the semi-arid grasslands.

Chapter 5 Effects of decadal geothermal warming on above- and belowground biomass distribution and production in a subarctic grassland

Fine root (i.e. roots with a diameter < 2 mm) growth accounts for roughly 20% of global terrestrial net primary production (McCormack et al., 2015) and is very sensitive to environmental changes, particularly to changes in temperature (Bai et al., 2010) and increasing CO_2 (Dieleman et al., 2012). Moreover, fine roots are the primary pathway for water and mineral nutrient uptake to support plant growth, therefore playing an important role in linking above- and belowground carbon processes and in controlling soil carbon cycling (Gao et al., 2008; McCormack et al., 2015; See et al., 2019). However, knowledge about how future warmer climate will affect fine roots is very limited, especially in cold ecosystems (Schwieger, Kreyling, Milbau, & Blume-Werry, 2018), and a better understanding of fine root production and its response to warming is crucial to understand terrestrial biogeochemical cycles in a future warmer climate (Rustad, 2008).

The effects of warming on fine root production are typically divergent in different ecosystems (Song et al., 2019), and have been found to be positive (Xiong et al., 2018), negative (Bai et al., 2010) or completely uninfluential (Schwieger et al., 2018). This lack of consistency may be attributed to the fact that fine root production can be influenced by warming in various direct and indirect ways regardless of the site-specific climate conditions (Song et al., 2019). For example, altered root-shoot ratio and root density, or altered water and nutrient availability, microbial enzymatic activities and aboveground production have all been suggested as determinants of fine root production (Dybzinski et al., 2019; Fortier et al., 2019; Jourdan et al., 2008; Ma et al., 2012; Peek et al., 2006; Rygiewicz & Andersen, 1994). Moreover, all these factors may act antagonistically or synergistically potentially causing the observed complex warming responses of fine root production. Therefore, it is critical to disentangle the mechanisms controlling the response of fine root production to future global warming.

The effects of increased soil temperature on ecosystems have been studied using various techniques, including infrared heater (Nijs et al., 1996; Xu et al., 2012), open top chambers (Fang et al., 2018; Fang et al., 2017; Maestre et al., 2013) and heating cables (Melillo et al., 2011; Pries et al., 2017). However, there is a lack of long-term warming studies on above- and belowground biomass distribution, despite the acknowledged importance of long-term ecosystem adaptation in the overall vegetation responses (Eisenhauer et al., 2012; Liu et al., 2018; Z. Ma et al., 2017; Sistla et al., 2013). Rising temperatures could increase nutrient availability and extend the growing season in mesic ecosystem (Chen et al., 2016), all potentially increasing plant productivities (Liu et al., 2017), thus we hypothesized that shoot and fine root production will increase with long-term soil warming in subarctic grasslands located in mesic region. According to the optimal partitioning theory, plants preferentially allocate additional biomass to the aboveground organs under more fertile soil (Kobe et al., 2010), causing a decrease in root-shoot ratio (Qi et al., 2019). Thus, we further hypothesized that the root-shoot ratio will decrease with long-term soil warming in subarctic grasslands.

In order to evaluate the effects of long-term soil warming on above- and belowground biomass distribution and production, we conducted a study across a gradient of geothermally warmed soil in a subarctic grassland in Iceland, located in a humid oceanic region. The specific objectives of this study were 1) to examine whether soil warming alters fine root and shoot production, 2) to examine whether soil warming alters the root-shoot ratio, and 3) to test contrasting control pathways of the response of fine root and shoot production to soil warming.

5.1 Materials and methods

5.1.1 Study area

The experiment was conducted in unmanaged grasslands at the ForHot research site in the Hengil geothermal area (Sigurdsson et al., 2016), 40 km east of Reykjavik, Iceland (64^{o}00’01”N, 21^{o}11’09”W; 83-168 m a.s.l.). The area has an oceanic climate, characterized by a mean annual air temperature of 5.2 ^{o}C, with July being the warmest (12.2 ^{o}C) and December the coldest (-0.1 ^{o}C) (Icelandic Meteorological Office; www.vedur.is). Mean annual precipitation and wind speed were 1460 mm and 6.6 m s^{-1}, respectively (Icelandic Meteorological Office; www.vedur.is). The soils were classified as Brown Andosols and had a silty-loamy texture (Sigurdsson et al., 2016). The grassland, which was unmanaged and fenced to protect from livestock grazing, is dominated by *Agrostis capillaris*, *Ranunculus acris* and *Equisetum pratense*, all perennial species with short aboveground parts that regrow each year from underground stem or rhizomes (N. I. W. Leblans et al., 2017).

The soil at the study site has been warmed since May 2008, when an earthquake shifted geothermal systems to previously unwarmed soil (Sigurdsson et al., 2016). Soil warming occurs via heat conduction from the underlying bedrock that is warmed by hot groundwater. Geothermal water remains confined within the bedrock and no signs of soil contamination by geothermal byproducts, such as exchangeable sulfur, were found. Since hot water intrudes the bedrock through faults, temperature gradients occur, with higher temperatures above the fault, and declining temperatures perpendicular to the fault. Further detailed description of the study site can be found in (Gargallo-Garriga et al., 2017; Holmstrup et al., 2018; Leblans et al., 2017; Marañón-Jiménez et al., 2018; Radujkovic et al., 2018; Sigurdsson et al., 2016; Walker et al., 2018).

5.1.2 Experimental design

Five transects were established in autumn 2012, with soil warming at 10 cm depth ranging from +0 °C to +20 °C, where six 2×2 m permanent plots were established along each transect at different level of warming (Sigurdsson et al., 2016). Soil temperature was recorded hourly at 10 cm soil depth in each permeant plot using TidbiT v2 HOBO® data loggers (Onset Computer Corporation, Bourne, Massachusetts, USA). Given the high workload associated with root studies, we selected three warming levels in each of the five transects. Soil temperatures in the selected plots were on average +0 °C, +3.7 °C and +7.6 °C above ambient (annual temperatures from July 2017 to July 2018 in the upper 10 cm of soil) (Table 5.1).

Table 5.1 Soil temperature in *situ* soil warming. Different letters indicate significant difference among warming treatments at $P < 0.05$ (one-way ANOVAs and Tukey-Kramer post hoc tests).

Transect	+0 °C	+3.7 °C	+7.6 °C
1	5.5	10.9	8.9
2	6.1	8.0	11.2
3	6.3	9.2	15.7
4	4.2	9.4	14.3
5	7.1	10.2	16.8
Mean annual soil T (°C)	5.8±1.0c	9.5±1.1b	13.4±3.3a

5.1.3 Soil properties

In July 2014, two soil cores in each plot were taken to a depth of 10 cm using an auger (4.6 cm inner-diameter), one to measure P, K^+, Ca^{2+}, Mg^{2+} and one to measure soil bulk density using a cutting ring (4.6 cm inner-diameter) (Kleibl, Klvac, Lombardini, Porhaly, & Spinelli, 2014). In July 2018, ten years after the onset of the soil warming, the soils were resampled to measure SOC, TN and soil bulk density. SOC and TN were determined by dry combustion with a Thermo Flash 2000 NC Analyser (Thermo Fisher Scientific, Delft, The Netherlands). A 7.5 g subsample of fresh soil was extracted with 0.5 M $NaHCO_3$ within 24 h of sampling, digested at 400 ºC with H_2SO_4 and selenium as a catalyst, and P was determined from the digested $NaHCO_3$ extracts (Marañón-Jiménez et al., 2019). Base cations (K^+, Ca^{2+}, Mg^{2+}) were measured using acid destruction with H_2SO_4, salicylic acid, H_2O_2 and selenium method (Courchesne, Turmel, & Beauchemin, 1996; Mautner, 1999). All analyses were performed by colorimetric detection with a San^{++} Continuous Flow Analyzer (Skalar Analytical B.V., Breda, Netherlands). Nutrient pools were calculated as the soil nutrients concentration multiplied by soil bulk density and soil depth.

5.1.4 Plant properties

In July 2018, above- and belowground plant biomass stocks were measured. Aboveground biomass was measured by clipping a 0.2×0.5 m area to the soil surface in each plot. In the same clipped area, two soil cores were taken to a depth of 15 cm using an auger (4.6 cm inner-diameter) to measure standing root biomass. Aboveground samples were sorted into vascular plants and mosses and dried to constant mass at 70 ºC. Oven dried vascular plant biomass is referred as shoot biomass and production hereafter. Roots were carefully washed by wet sieving in a 0.15 mm mesh under gently flowing water to remove attached soil and were subsequently dried to constant mass at 70 ºC to get root standing biomass.

Fine root production ($g\ m^{-2}$ or tons per ha^{-1}) was measured by root mesh method (Hirano et al., 2009). In April 2018 (growing season starts in late May) (Leblans et al., 2017), in each plot two root meshes with 2 mm mesh size, 10 cm length and 7.5 cm width were vertically inserted down to 10 cm depth in the soil using a straight stainless steel blade with 10 cm width, 20 cm length and 2 mm thick. At the end of September in 2018 (growing season stops in August), a soil volume of 3 cm thick (1.5 cm on either side of the mesh that was located in the middle), 7.5 cm wide and 10 cm deep, i.e. where the net was located in the middle, was taken out using two sharp straight and perching stainless steel blades with 20 cm length and 7.5 cm width which could easily cut off roots in soil. The collected soil volume was processed to get wet root samples like mentioned above. About 0.03g (dry mass) fine roots randomly chosen from the wet root samples in each plot were scanned to calculate root area and then dried to constant mass at 70 ºC. The specific root area (SRA) was calculated as dividing the root area by the dried biomass (Lõhmus, Oja, & Lasn, 1989). Meanwhile, the other root were also dried to constant mass. The total dry mass of all roots that grew through the mesh from April to September was considered as the annual fine root production. Root turnover was calculated as the ratio of fine root production versus standing root biomass.

5.1.5 Statistical analysis

Prior to statistical analysis, we assessed the data quality with Dixon's Q test to identify and remove outliers (DIXON, 1950; Efstathiou, 2006; Onoz & Oguz, 2003) and produced the final dataset (Table 5.2 and 5.3). Then a general linear model was applied to test the effects of soil warming on standing root biomass, fine root and aboveground production, and root-shoot ratio, with soil warming as a fixed variable and transect as a random variable. Tukey-Kramer post-hoc tests were applied due to the unequal sample sizes among treatments after removing outliers, to determine the differences among treatments. Data were transformed when required to ensure normality and homoscedasticity (Quinn & Keough, 2009). Structural equation modeling (SEM) was performed using AMOS 21.0 to quantify the relative importance of the potential direct and indirect pathways in mediating the soil warming effects on fine root and shoot production based on significance of the regression equation (Pearson's correlation analysis), conceptual modeling (Fig. A), the goodness of model fit and logical reasoning. All statistical analyses were performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

Table 5.2 Data amount of variables in analyzing difference among treatments.

Treatment	Shoot production	Standing Root biomass	Fine root production	T	SWC	RS	SOC	TN	P	K+	Ca+	Mg+	SRA
+0 ºC	3	5	4	5	5	3	5	5	3	3	3	3	5
+3.7 ºC	4	5	5	5	5	4	4	4	4	4	4	4	4
+7.6 ºC	4	5	5	5	5	4	4	5	4	4	4	4	5

Table 5.3 Data amount of variable combinations in analyzing relationship between biomass and environment factors.

Variables	T	SWC	SOC	TN	SRA
Shoot biomass	11	11	10	11	10
Standing root biomass	15	15	13	14	14
Fine root production	14	14	12	13	13

5.2 Results

5.2.1 Effects of soil warming on soil water and nutrient pools

Soil gravimetric water content was significantly lower under warming treatments than in the control treatment (Fig. 1B), even though no significant difference in soil volumetric water content was observed between these treatments (Fig. 1A) due to the different soil texture (data not shown here). SOC and TN pools were significantly lower in the warming treatments than in the control treatment, but no significant differences were observed between the +3.7 ºC and +7.6 ºC treatments (Fig. 1C and D). There were no significant differences in TP, K^+, Ca^{2+} and Mg^{2+} among any treatments (Fig. 1E-H).

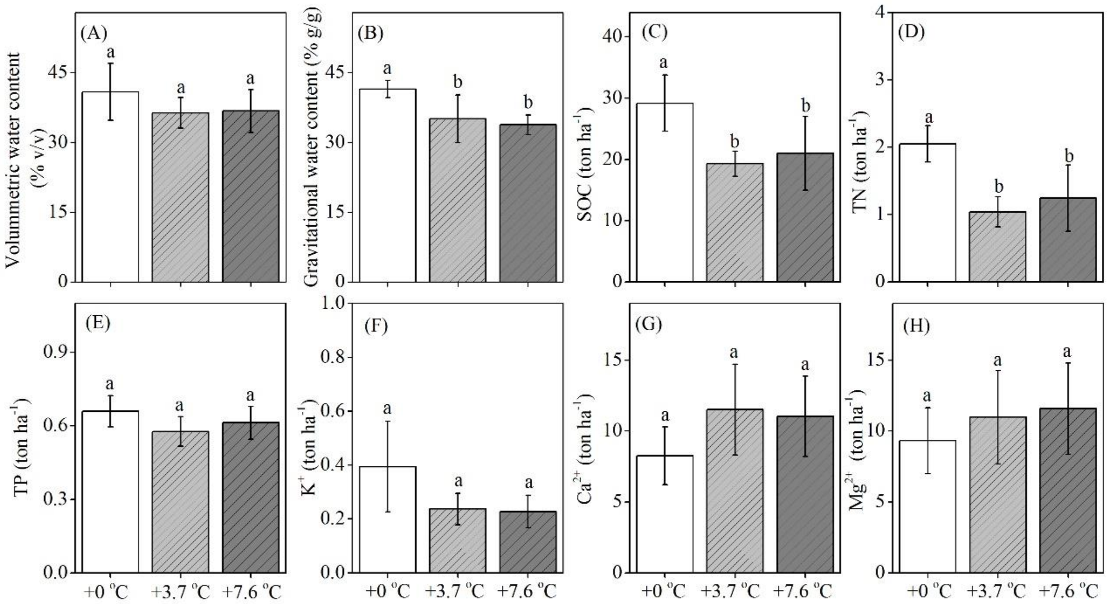


Fig. 5.1. Soil water and nutrient pools under +0 ºC, +3.7 ºC and +7.6 ºC treatments. Different letters represent significant difference at $P < 0.05$.

5.2.2 Effects of soil warming on above- and belowground production and root traits

Fine root and shoot production were significantly lower in warming treatments than under ambient temperature conditions, with the lowest fine root production in the +7.6

°C treatment ($P<0.05$, Fig. 5.2). However, there were no statistically significant differences in standing root biomass and root-shoot ratio among the three treatments ($P > 0.05$, Fig. 5.3A and C). Fine root turnover rate was significantly lower under +7.6 °C than under +3.7 °C, whereas no significant difference was observed between the two warmed and the control treatment (Fig. 5.3B). SRA was significantly higher under warming treatments than in the ambient temperature treatment, with the highest value at +7.6 °C (Fig. 5.3D). As a result, although the plant community in warmed plots produced lower fine root biomass, the total absorbing surface did not decline through a compensating increased SRA.

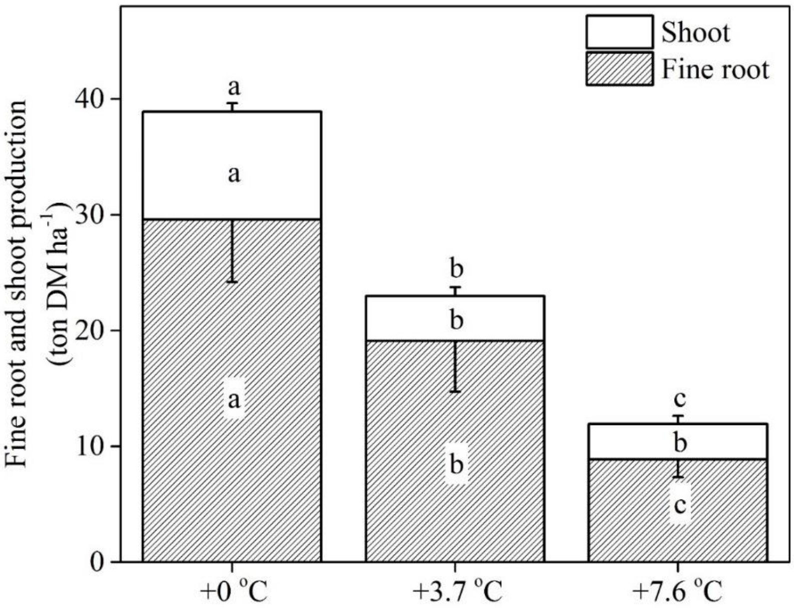


Fig. 5.2 Fine root production and shoot biomass under +0 °C, +3.7 °C and +7.6 °C treatments. Different letters represent significant difference at $P < 0.05$.

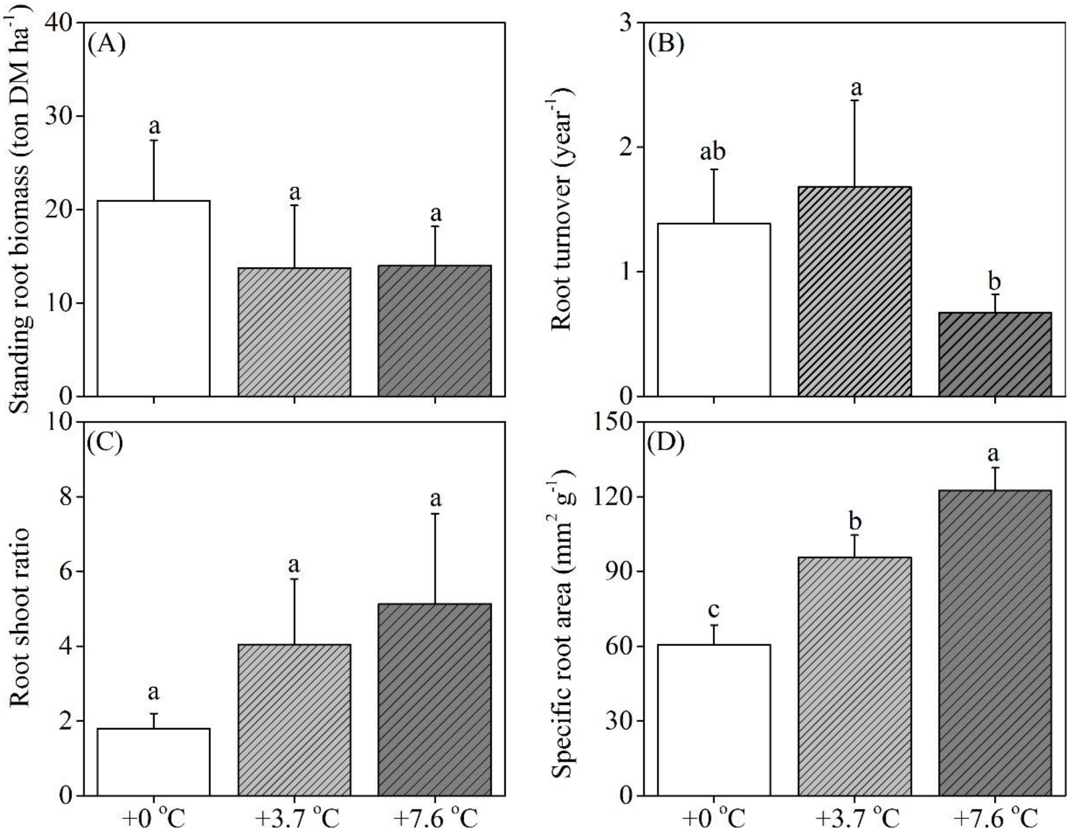


Fig. 5.3 Standing root biomass (A), root turnover (B), root-shoot ratio (C), and specific root area (SRA) (D) under +0 °C, +3.7 °C and +7.6 °C treatments. Different letters represent significant difference at $P < 0.05$.

5.2.3 Relationships of above- and belowground biomass and production with SRA, soil temperature, water content and nutrient pools

Shoot biomass (and thus production) and fine root production were significantly negatively correlated with mean annual soil temperature and SRA ($P < 0.05$, Fig. 5.4A, E, K and O) and significantly positively correlated with SWC, SOC and TN pools ($P<0.05$, Fig. 5.4B-C and L-N). Standing root biomass exhibited significant relationships only with soil temperature (negative correlation) and with soil moisture (positive correlation) ($P<0.05$, Fig. 5.4F and G), but not with SOC, TN, or SRA ($P>0.05$, Fig. 5.4H-J).

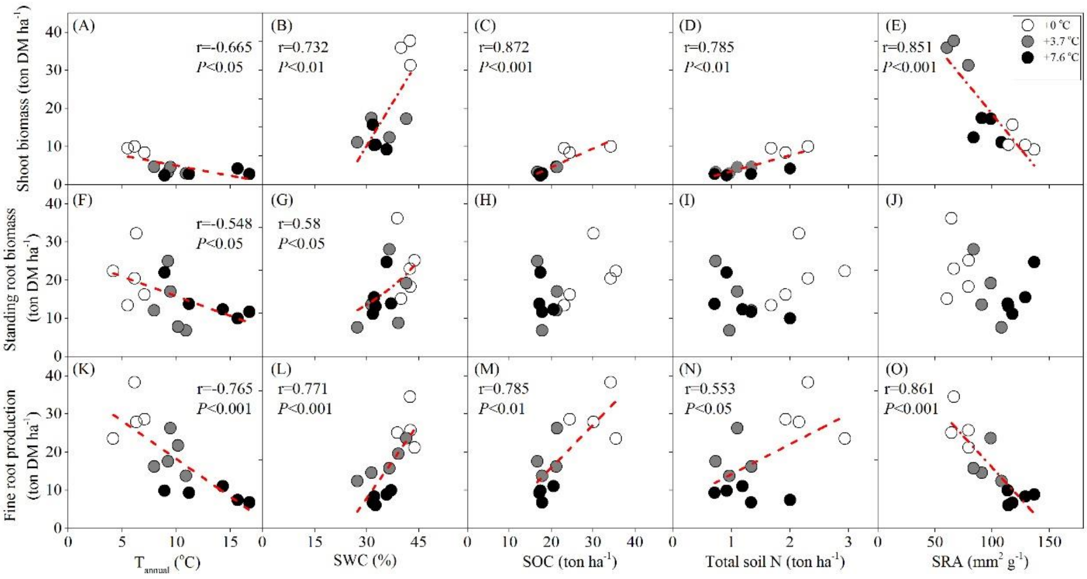


Fig. 5.4 Relationships of above- and belowground biomass and production with SRA, soil temperature, water content and nutrients pool. Significance is at $P < 0.05$.

5.2.4 Structural equation modeling of shoot production and fine root production

The final SEM of shoot production showed that T, both directly and indirectly, affected shoot production through regulating SWC and substrate pools (i.e. SOC and N). Together, the soil N pool was the most important predictor shaping the variation in shoot production. These abiotic variables explained 95% of the variance of shoot production (Fig. 5.5).

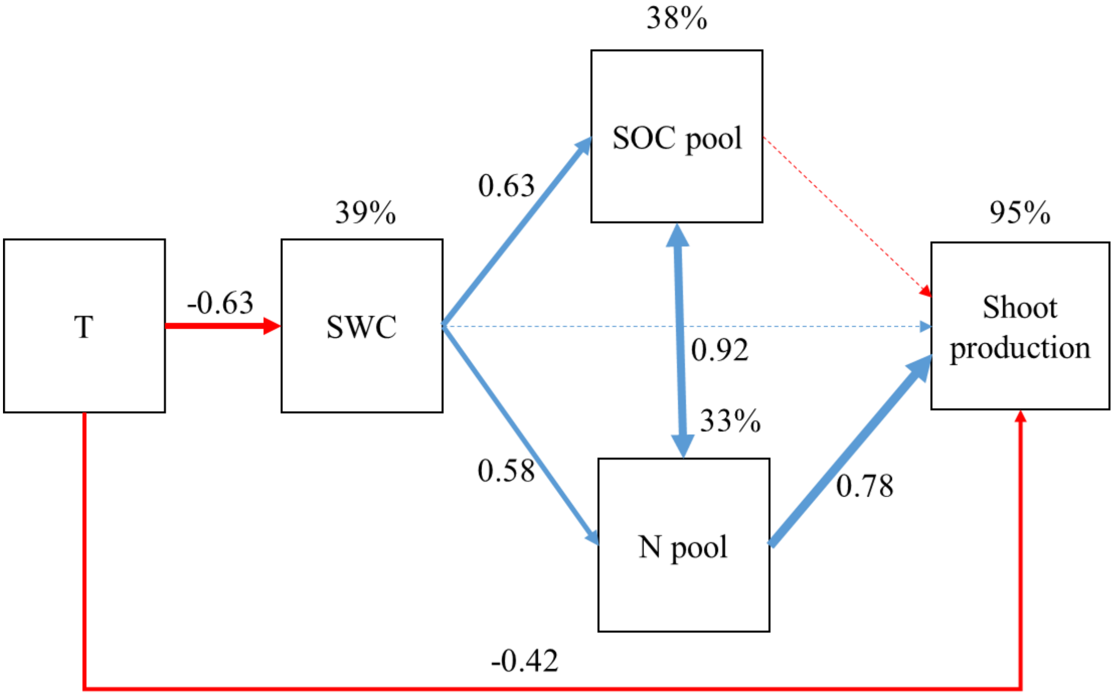


Fig. 5.5 Structure equation modelling (SEM) with variables (boxes) and potential causal relationships (arrows) for shoot production ($\chi^2 = 1.282$, df = 2, $P = 0.527$, RMSEA < 0.001). Single headed arrows represent the hypothesized direction of causation. Numbers next to single headed arrows are standardized path coefficients, which indicate the effect size of the relationship. The double headed arrow represents the covariance between related variables. Red and blue arrows indicate positive and negative relationship. The proportion of variance explained appears above each response variables in the model. Solid line means significance and dash line means non-significance. Soil organic matter reflects soil fertilizer status but is difficult to measure, which can be instead by SOC (Kopittke et al., 2019; Schmidt et al., 2011). Thus SOC was used as the soil fertilizer indicator in the final SEM.

In contrast to aboveground production, the SEM analysis suggested that T only indirectly affected fine root production through regulating SWC, SOC and TN, but also by affecting biotic factors (shoot production and SRA) (Fig. 5.6). Taking the direct and indirect effects together, the TN pool was the most important predictor shaping the variation in fine root production. Combined, these variables explained 95% of the variance in fine root production (Fig. 5.6). Specifically, SOC and SRA had direct effects on fine root production, whereas SWC had only indirect effect by mediating SOC and TN pools and both biotic factors (shoot production and SRA). SEM thus detected the direct and indirect effects of shoot production and TN pool on the variation in fine root production.

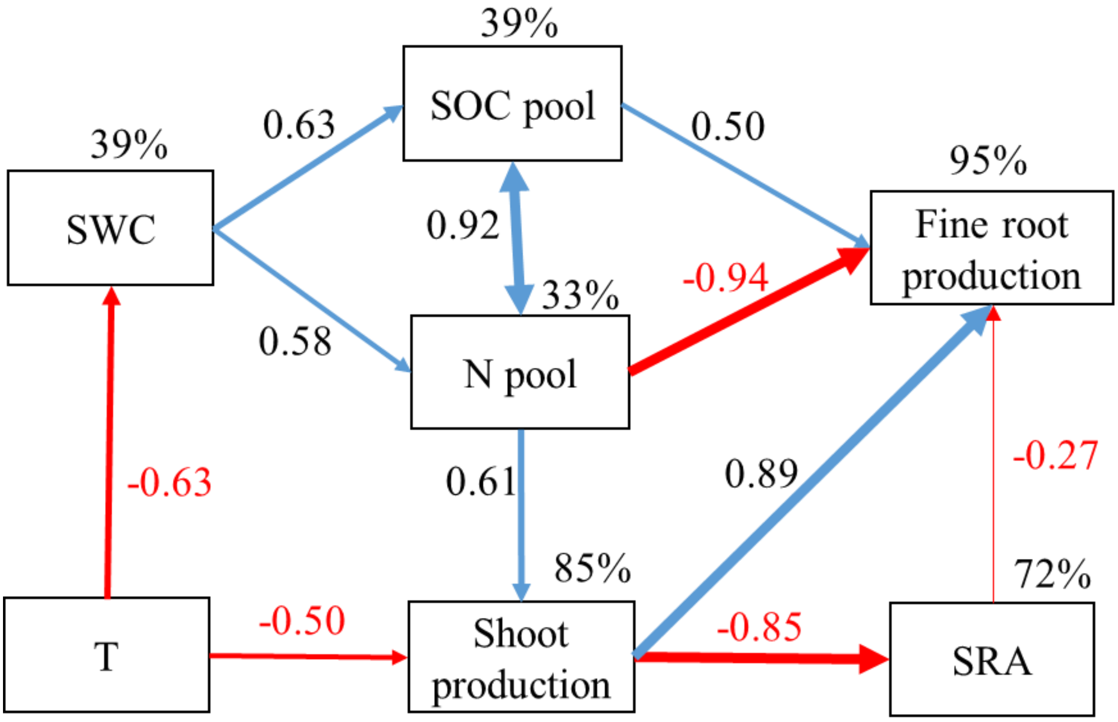


Fig. 5.6 Structure equation modelling (SEM) with variables (boxes) and potential causal

relationships (arrows) for fine root production ($\chi^2 = 0.215$, df = 1, $P = 0.643$, RMSEA < 0.001). Single headed arrows represent the hypothesized direction of causation. Numbers next to single headed arrows are standardized path coefficients, which indicate the effect size of the relationship. The double headed arrow represents the covariance between related variables. Blue and red arrows indicate positive and negative relationship. The proportion of variance explained appears above each response variables in the model.

5.3 Discussion

This study tested two hypotheses 1) shoot and fine root production increase with long-term soil warming because of accelerated nutrient cycling (Noyce, Kirwan, Rich, & Megonigal, 2019; Schaeffer, Sharp, Schimel, & Welker, 2013), and 2) the root-shoot ratio decreases with long-term soil warming (optimal partitioning theory). We found that the increase in temperature did not change standing root biomass and root-shoot ratio, but reduced above- and belowground production (Figs. 5.2 and 5.3). Results thus support the rejection of both hypotheses; i.e. we have witnessed a positive ecosystem feedback to the decade long warming in this sub-arctic grassland that has decreased its carbon storage both aboveground and belowground.

Both responses seem to indicate that the sub-arctic grassland ecosystem was more affected by a warming-induced change in nutrient availability than the warming per se. In this study, soil warming increased SRA (Fig. 5.3D) which could be partly explained by the observed reduction in the soil N pool (Fig. 5.1D). Reduced soil N pool implied that plants were growing in a soil with reduced N availability, as found by (Marañón-Jiménez et al., 2019) for the same site. It has been suggested that in response to reduced N availability fine roots produce a larger absorbing area per unit root biomass. Previous studies also suggest that under low-nutrient environment, the survival strategy adopted by plant is to extend root to produce a larger soil resource area for fine root and invest less unit carbon in fine root to obtain as much as soil resources (Jackson, Mooney, & Schulze, 1997; Z. Ma et al., 2018; Reich & Cornelissen, 2014), thus producing more coarse roots (diameter > 2 mm) and fine root absorbing area for exploring a larger volume of soil (Leppälammi-Kujansuu et al., 2012; Reich & Cornelissen, 2014). Furthermore, increased soil temperature did not change standing root biomass but reduced fine root production (Figs. 5.2 and 5.3A), implying that either fine root longevity increased, or that the coarse root biomass increased, which is what we will investigate in the next study.

Previous studies have shown that increased temperature often increases aboveground production (Fang et al., 2018; Sherry et al., 2008; Wan, Hui, Wallace, & Luo, 2005; Xia & Wan, 2013), mainly by extending the growing season (Fang et al., 2018; Wan et al., 2005; Xia & Wan, 2013), promoting photosynthesis (Lewis, Lucash, Olszyk, & Tingey, 2011), or increasing nutrient availability (Sherry et al., 2008). In this study, we observed that shoot production was significantly decreased by the long-term warming (Fig. 5.2). But still the total photosynthesis (carbon input) might have been increased since the integrated seasonal NDVI was observed to increase at higher temperatures (Leblans et al., 2017). In Iceland, nitrogen is assessed to limit grassland productivity (Niki I. W. Leblans et al., 2017; Leblans et al., 2014). Hofer et al (2017) have shown that nitrogen limitation and decreased water availability could inhibit plant growth, mainly by reducing photosynthetic rate (Hofer, Suter, Buchmann, & Lüscher, 2017). In this study, long-term soil warming induced soil water reduction (but never approaching wilting point) and substantial losses of SOC and TN (Fig. 5.1A and B, and Marañón-Jiménez et al., 2019), which may explain the decrease of aboveground productivity.

Previous studies have attributed the effects of increased temperature on fine root production to different controls. For example, Xiong et al. (2018) found that increased temperature increased fine root production in boreal forest, mainly due to water stress induced by increased temperature, forcing plants to allocate more C to root growth to improve water acquisition (Xiong et al., 2018). Leppälammi-Kujansuu et al. (2012) showed that increased temperature increased soil nutrient availability, which promoted both above- and belowground production (Leppälammi-Kujansuu et al., 2012). Johnson et al. (2006) showed that fine root productivity did not respond to temperature increase, which they attributed to low nitrogen availability considering the fine root response to temperature increase (Johnson et al., 2006). Meanwhile, Ma et al. (2012) showed that aboveground biomass had a significant positive relation with fine root production in a tree species (Ma et al., 2012). In our study, SEM suggested that the decrease in fine root production under warming was mainly attributed to the reduced N pool and aboveground productivity (Figs. 5.5 and 5.6). While warming may have enhanced soil N availability in the short term, after 10 years the clear N losses that go hand-in-hand with higher mineralization rate, resulted in depleted soil N pool, suggesting that warming had reduced N availability (Marañón-Jiménez et al., 2019). The SEM also suggested that increased temperature indirectly reduced soil nitrogen pool by reducing soil moisture, thus promoting fine root production. Decreased aboveground production, induced indirectly by increased temperature and its effect on SOC and TN pools, also decreased fine root production. Moreover, the morphology of fine roots structure was altered in warmer soil, higher SRA of roots indicates larger root-soil contact area favoring nutrient uptake and compensating part of the decreased fine root production. Consequently, the balance between these positive and negative driving pathways resulted in an observably decreased fine root production. These findings revealed that the response of fine roots to soil warming resulted from multiple mechanisms driven by both abiotic and biotic factors.

Biomass allocation is an important indicator of soil resource status (Klimešová, Martínková, Ottaviani, & Field, 2018), and is well represented by the root-shoot ratio (Wilson, 1988). The root-shoot ratio reflects the differential investment of photosynthates between the above- and belowground organs (Xu et al., 2012). According to the optimal resource partitioning theory (Bloom, Chapin, & Mooney, 1985; Kobe et al., 2010), plants will allocate more photosynthates to aboveground organs in resource-rich soil, and more to belowground organs in resource-poor environments. However, plant biomass allocation is also regulated by other environmental factors, such as temperature (Liu et al., 2018; Xu et al., 2012), which is an important limiting factor for plant growth in cold ecosystems (Sistla et al., 2013). Increased soil temperatures could thus have induced a more benign soil thermal environment for vegetation in this subarctic grassland (Table 5.3). However, soil warming resulted in significant losses of SOC and TN pools (Fig. 5.1), thus inducing a nutrient-poor environment for the vegetation. Bai et al. (2010) suggested the observed responses of plant C allocation to warming resulted from the balance between favorable and unfavorable environments (Bai et al., 2010). In our study, the higher thermal environment may have favored aboveground grass growth, while the reduced N pool antagonized this effect, finally yielding no changes in root-shoot ratio in response to soil warming. This study did not allow distinguishing the thermal effect from the nutrient on plant biomass allocation which remains to be investigated in the future.

5.4 Summary

Our in *situ* study provided unique data for investigating the responses of below- and aboveground biomass distribution to long-term soil warming. Our results showed that long-term soil warming did not change standing root biomass, but decreased aboveground biomass and production. Decreased fine root production induced by soil warming resulted from decreased aboveground biomass and soil nutrient pools, suggesting an urgent need to improve our understanding of the driving mechanism in response of fine root to long-term soil warming to test and validate carbon models.

Chapter 6 Conclusions

6.1 Main conclusions

In this thesis, we investigated 1) the responses of soil respiration to warming and nitrogen addition and 2) the responses of soil microbial properties to warming in two typically semi-arid grassland ecosystems; and 3) the responses of root biomass and photosynthates to decadal soil warming in a subarctic grassland ecosystem. These studies could provide important parameters and model validation for simulation of key soil carbon processes in climate-sensitive grassland ecosystems and terrestrial ecosystem processes in response to climate change.

6.1.1 Seasonal responses of soil respiration to warming and nitrogen addition

In the alfalfa grassland ecosystem (Chapter 2), the soil respiration rate showed similar seasonal dynamics with temperature and precipitation with peak values in the month with the highest temperature and the most precipitation. In the wet year, the inhibition of soil respiration by warming occurred less frequently than in the dry year. Warming significantly increased soil temperature, but decreased soil moisture and vegetation coverage during the growing seasons, and had no significant effect on the daily change of soil moisture during the non-growing season. Therefore, the response of soil respiration to warming during the growing season was suppressed, whereas showed an increase in the non-growing season. Nitrogen addition accelerated the utilization of soil nitrogen and thus promoted soil respiration. The combination of warming and nitrogen addition reduced the temperature sensitivity of soil respiration.

6.1.2 Responses of soil respiration and its components to warming and nitrogen addition

In the fenced grassland ecosystem (Chapter 3), warming significantly increased soil temperature but reduced soil moisture. Meanwhile, warming advanced the plant growing season by 4 days and increased the plant aboveground biomass. Soil respiration rate had significant positive correlations with aboveground biomass, and soil temperature and moisture. Warming significantly reduced total soil respiration and heterotrophic respiration, but did not change autotrophic respiration. Nitrogen addition significantly stimulated autotrophic respiration but reduced heterotrophic respiration, resulting in an insignificant response of total soil respiration to nitrogen addition. The effects of warming and nitrogen addition on total soil respiration did not show a significant interaction effect. The effects of warming and nitrogen addition on soil heterotrophic respiration showed a synergistic effect, resulting from the decoupling between the antagonistic effect on autotrophic respiration and the synergistic effect on heterotrophic respiration.

6.1.3 Responses of soil microbial communities to warming

In the fenced grassland ecosystem (Chapter 4), warming significantly increased soil temperature and plant aboveground biomass, and reduced soil moisture and pH. Soil microbial respiration rate was significantly positively correlated with soil moisture and pH, and negatively correlated with soil inorganic nitrogen content. 2-year OTC warming did not change the soil microbial community structure and microbial biomass but significantly reduced the microbial respiration rate. The decrease of soil microbial respiration rate caused by temperature increase is the result of the joint regulation of soil temperature, moisture, inorganic nitrogen, pH and plant aboveground biomass. These results indicate that the response of microbial community structure and microbial biomass to warming is not significant due to the adaptation of the microbial community to short-term warming of 2 ^{o}C.

6.1.4 Responses of plant root biomass and photosynthates distribution to long-term soil warming

In the subarctic grassland ecosystem (Chapter 5), plant aboveground biomass and fine root productivity were significantly positively correlated with soil carbon and nitrogen pools and soil moisture ($P < 0.05$), and significantly negatively correlated with soil temperature and specific root area ($P < 0.05$). Long-term soil warming did not cause the loss of soil base ions, but significantly reduced the soil carbon and nitrogen pools and increased the specific root area. Long-term soil warming reduced aboveground biomass and fine root productivity, whereas had no significant effect on the root shoot ratio with a slightly increasing trend. Together, soil temperature and moisture, carbon and nitrogen pools, aboveground biomass, and specific root area accounted for 95% of the changes in fine root productivity. Our results implies that the responses of subarctic grassland ecosystems to climate warming is jointly regulated by multiple environmental factors, but the regulation of soil nutrients plays a leading role.

6.2 Research deficiencies and prospects

6.2.1 Coupling among multiple warming techniques

OTC could reduce the air flow inside the plot, such as wind speed. The micro-trenching method could affect the substrates required for the growth of microorganisms in the soil. How these factors affect the responses of the key soil carbon processes to climate change is not well understood in this study. In addition, the ways that OTC and natural geothermal heating the soil are very different, that is, OTC warms the soil from top to bottom, and geothermal heats the soil from bottom to top. Thus soil respiration and plant growth may respond differently to the two warming methods, which is still lacking understood in our research. Therefore, we should adopt more methods to further verify the research results when conducting *in situ* manipulative experiments.

6.2.2 Long-term experiments

In this study, our investigations on soil respiration and microbial communities are both short-term, which is of great value to our understanding of the short-term impact of climate change on ecosystems. However, we have not yet been able to reveal the response mechanisms of key soil carbon processes in the long-term climate change. The response of soil respiration to climate change has obvious inter-annual differences, that is, climate warming is more likely to inhibit soil respiration in semi-arid regions in dry years. In addition, climate change is a long-term process, and its impact on ecosystems will also be on a long-term scale. Therefore, it is very necessary to carry out long-term observations of similar *in situ* manipulative experiments when conditions permitted.

6.2.3 Coupling in situ manipulative experiments with model simulations

In this study, we divided the two-year study of the alfalfa grassland ecosystem into a dry year and a humid year, each of which is only one year, and the representativeness of the results needs to be further verified. The simulation of the model provides the possibility for this verification. Therefore, it is necessary to carry out the combination of *in situ manipulative* experiment and model simulation.

6.2.4 *In situ* manipulative experiments with multiple factors

In this study, the *in situ* manipulative experiments only included one or two global drivers. Most of the current studies also focus on the interaction of one or two global factors. However, the responses of ecosystem to global change is the result of the coupling of multiple global factors. Therefore, it is more meaningful to focus on establishing multi-factor comprehensive manipulative experiments for predicting the impacts of future global changes on the ecosystem.

6.2.5 Study of extreme climate events

The frequency of extreme climate events will increase in the future. The drought caused by warming in this study could override the promotion of warming on soil respiration. The maximum value of soil respiration rate occurred in the month with the highest precipitation. Both of them indicate that extreme climate events may regulate the responses of terrestrial ecosystems to climate change. Therefore, it is necessary to establish the study of extreme climate events in climate-sensitive areas.

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Appendix

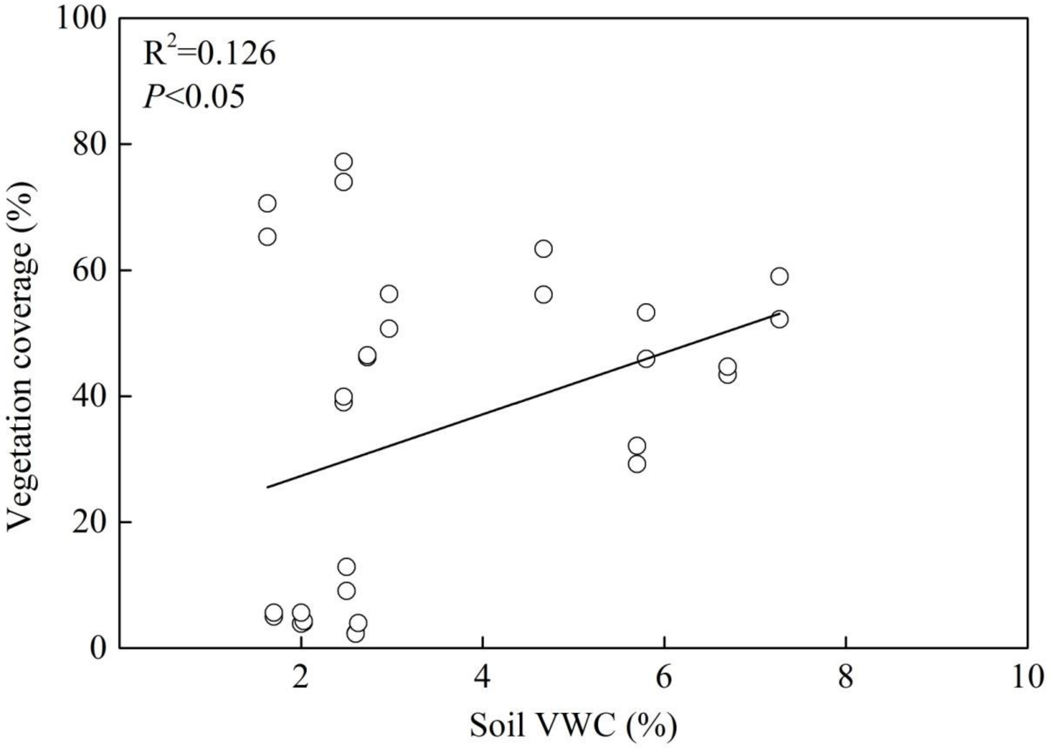

Fig. A1 Relationship between vegetation coverage and soil volumetric water content. Each point represents each observation (the mean value of three replicates) every measurement time in growing season of 2014.

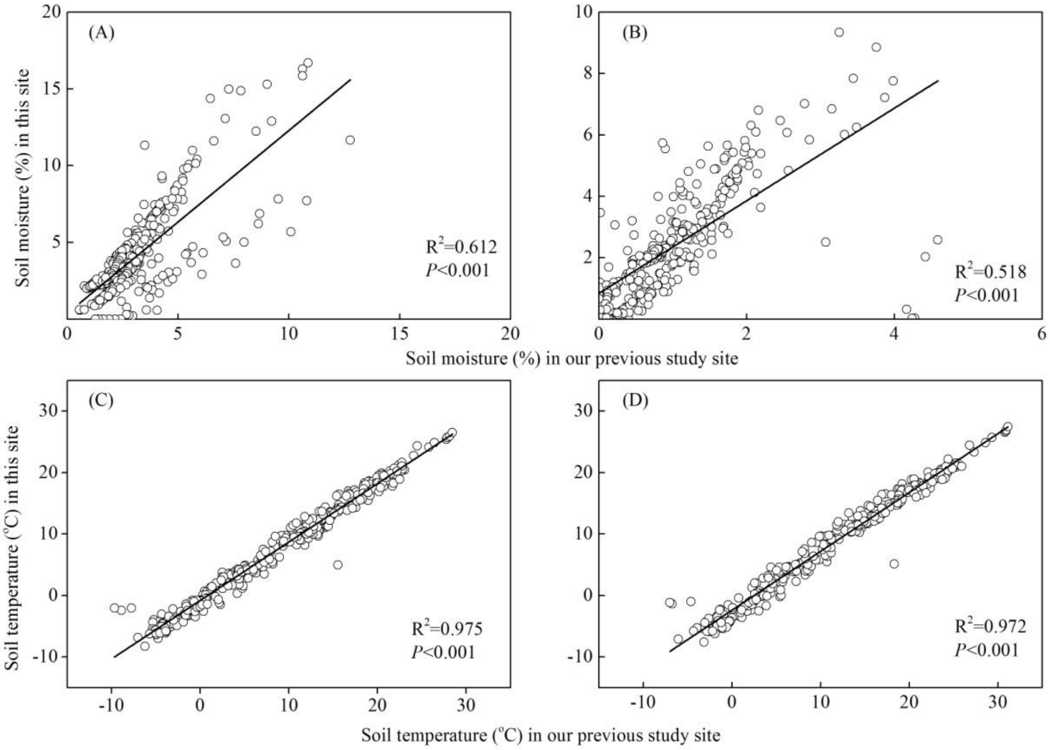

Fig. A2 The comparisons of soil moisture in control (a) and warming (b) treatments and soil temperature in control (c) and warming (d) treatments between this study and our previous study (Fang et al. 2017).

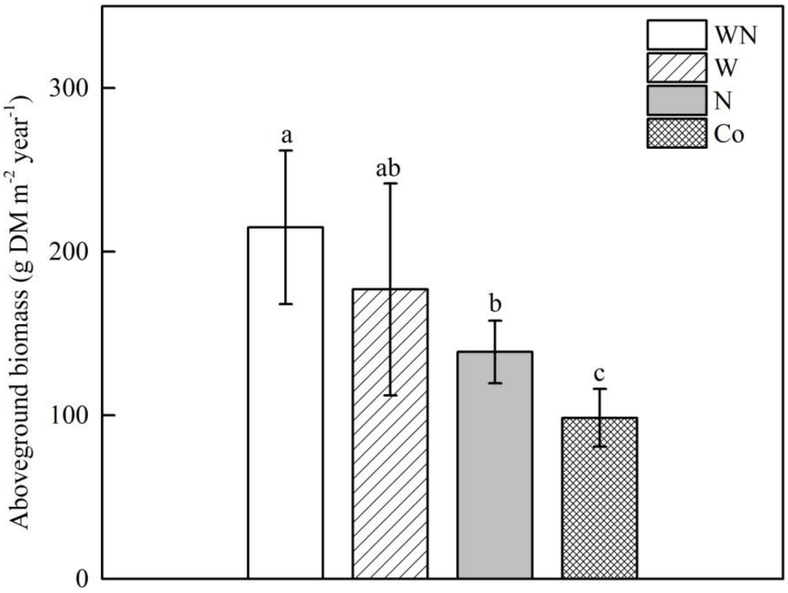

Fig. A3 Aboveground biomass (AGB) in 2016 under control (Co), nitrogen addition (N), warming (W), warming and nitrogen addition (WN). Different letters represent significant difference at $P < 0.05$. Data shown are means ± standard deviation.

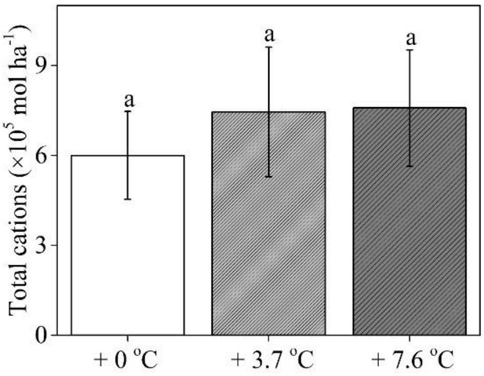

Fig. A4 Total soil cations pool under +0 ºC, +3.7 ºC and +7.6 ºC treatments. Different letters represent significant difference at *P* < 0.05.

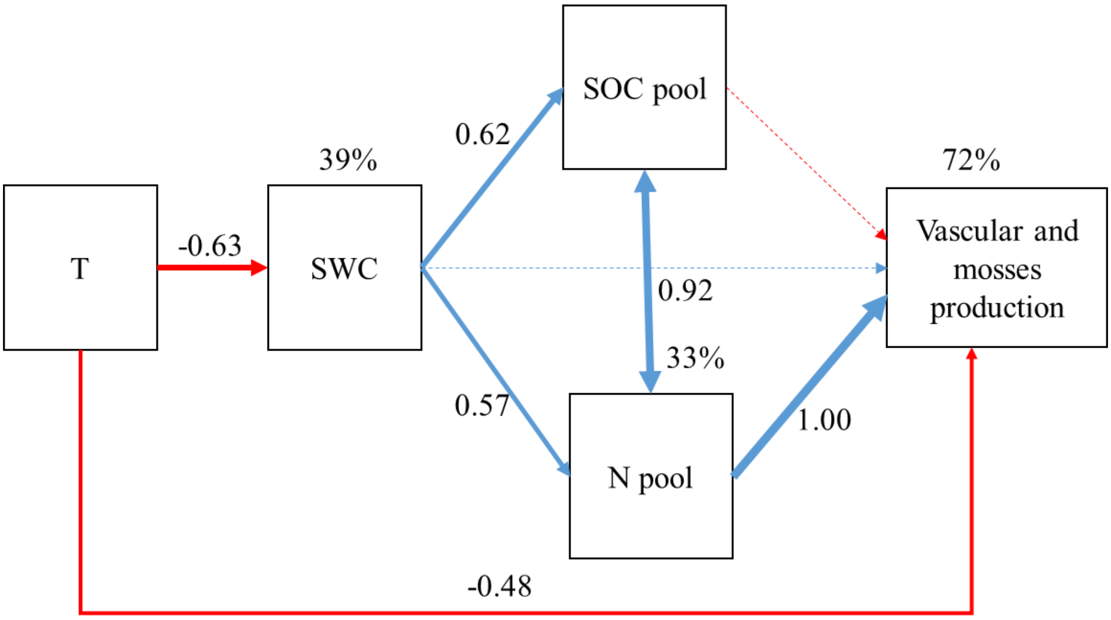

Fig. A5 Structure equation modelling (SEM) with variables (boxes) and potential causal relationships (arrows) for total aboveground vascular plant and mosses production ($\chi^2 = 1.339$, df = 2, $P = 0.512$, RMSEA < 0.001). Single headed arrows represent the hypothesized direction of causation. Numbers next to single headed arrows are standardized path coefficients, which indicate the effect size of the relationship. The double headed arrow represents the covariance between related variables. Red and blue arrows indicate positive and negative relationship. The proportion of variance explained appears above each response variables in the model.

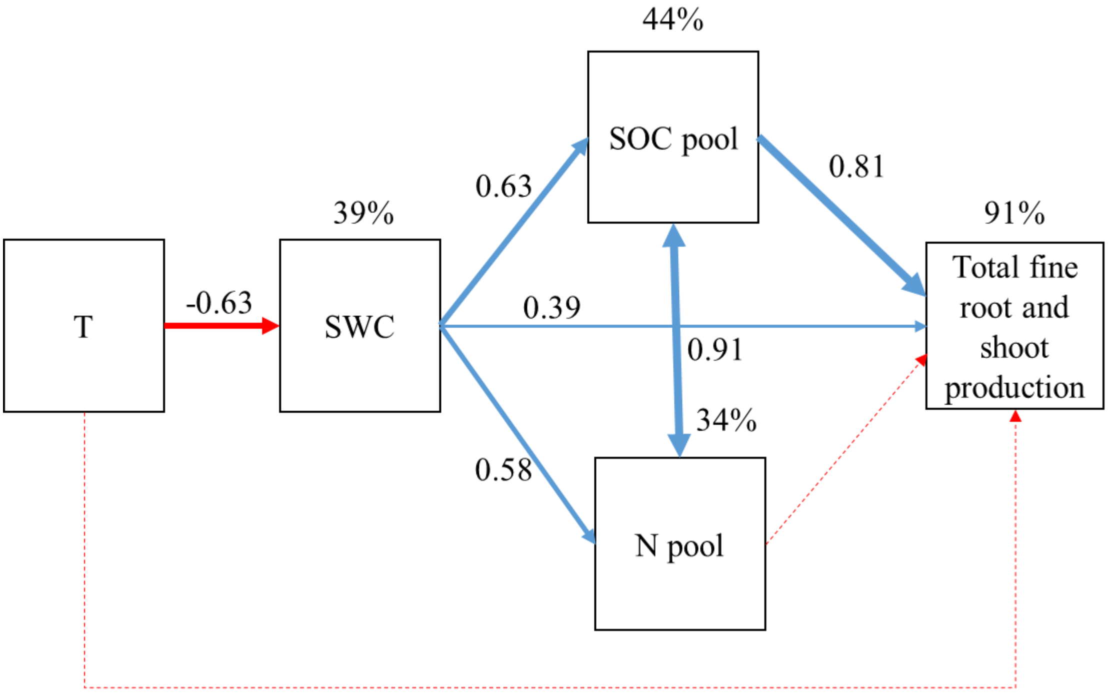

Fig. A6 Structure equation modelling (SEM) with variables (boxes) and potential causal relationships (arrows) for total fine root and shoot production ($\chi^2 = 2.64$, df = 2, $P = 0.267$, RMSEA < 0.151). Single headed arrows represent the hypothesized direction of causation. Numbers next to single headed arrows are standardized path coefficients, which indicate the effect size of the relationship. The double headed arrow represents the covariance between related variables. Red and blue arrows indicate positive and negative relationship. The proportion of variance explained appears above each response variables in the model.

Table A1 The curve fitting for biomass and height of different plant species

Plant species	Power equation	R^2
heteropappus altaicus	$0.001H^{1.7937}$	0.874^{**}
stipa	$0.0002H^{1.6955}$	0.978^{**}
Androsace erecta	$0.0007H^{1.9049}$	0.883^{*}
Artemisia capillaries	$0.0006H^{1.9268}$	0.934^{**}
Agropyron cristatum	$0.0002H^{2.1323}$	0.967^{**}
Setaria viridis	$0.0031H^{1.2037}$	0.946^{**}

Before the field experiment, the relationship between plant height and above-ground biomass was constructed. The height and biomass of per unit plant (PUP, defined as a bifurcation of plant coming up soil surface) was measured in each plot. Then we regressed the curve fitting between biomass and height of PUP for different species (Table A1).

Table A2 The beginning date of growing season under warming treatment (W) and control treatment (Co) in 2016

Year	W	Co
2016	March 25	March 29

The beginning of growing season was defined as the date when air temperature of continuous five days was higher than or equal to 5 °C in the first half year of 2016.

Curriculum vitae

Chao Fang was born on 3rd July 1989 in Chibi, where one famous war Battle of Chibi broke out during Three Kingdoms Period in China. In July 2012, He graduated from China University of Geosciences (Wuhan) with a bachelor degree in biological science and technology. In September 2012, he was admitted to science of Ecology, School of Life Sciences, Lanzhou University, and obtained a master's degree in July 2015. In September 2015, he was transferred to a MD-PhD program. Since December 2017, he was fully funded by the National Scholarship Council to go to study in PLECO at University of Antwerp in Belgium for two years, and returned to China in December 2019. During his PhD study period, he was awarded the title of Merit Postgraduate in Lanzhou University for four times from 2013 to 2017. He was awarded Yan Hanxi Career Enhancement Award in the 7th International Young Ecologist Forum in 2017. He won the First Prize of Excellent Paper and the Excellent Report in the graduate student academic annual meeting of school of life science at Lanzhou University in 2016 and the First Prize of Excellent Paper in 2018. He was awarded the national scholarship for doctoral students twice in 2017 and 2018, respectively. He was awarded Wang Dong Grass-Science Scholarship by the Chinese Grassland Society in 2018. He was awarded the first prize of outstanding academic graduate student by the State Key Laboratory of Grassland Agro-ecosystems in 2018. He is currently a reviewer for European Journal of Agronomy, Science of the Total Environment, and Environmental Pollution.

Research output

1. **Chao Fang**, Fengmin Li, Jiuying Pei, Jiao Ren, Yanhong Gong, Ziqiang Yuan, Wenbin Ke, Yang Zheng, Xiaoke Bai, Jiansheng Ye*. 2018. Impacts of warming and nitrogen addition on soil autotrophic and heterotrophic respiration in a semi-arid environment. Agricultural and Forest Meteorology. 2018, 248, 449-457. DOI: 10.1016/j.agrformet.2017.10.032. (IF2018=4.183)
2. **Chao Fang**, Jiansheng Ye*, Yanhong Gong, Jiuying Pei, Ziqiang Yuan, Chan Xie, Yusi Zhu, Yueyuan Yu. 2017. Seasonal responses of soil respiration to warming and nitrogen addition in a semi-arid alfalfa-pasture of the Loess Plateau, China. Science of the Total Environment. 2017, 590-591, 729-738. DOI: 10.1016/j.scitotenv.2017.03.034. (IF2018=5.589)
3. **Chao Fang**, Wenbin Ke, Matteo Campioli, Jiuying Pei, Ziqiang Yuan, Xin Song, Jian-sheng Ye*, Fengmin Li*, Ivan A. Janssens.2020. Unaltered soil microbial community composition, but decreased metabolic activity in a semi-arid grassland after two years of passive experimental warming. Ecology and Evolution. 2020. in revision.
4. Effects of decadal soil warming on above- and below-ground biomass distribution and production in a subarctic grassland. (To be submitted)
5. Ziqiang Yuan, **Chao Fang**[1], Rong Zhang, Fengmin Li*, Muhammad Mansoor Javaid, Ivan A. Janssens. 2019. Topographic influences on soil properties and aboveground biomass in lucerne-rich vegetation in a semi-arid environment. Geoderma. 2019, 344, 137-143. DOI: 10.1016/j.geoderma.2019.03.003 (IF2018=4.336)
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7. Jian-Zhou Wei, Kai Zheng, Feng Zhang, **Chao Fang**, Yu-Yu Zhou, Xue-Cao Li, Feng-Min Li, Jian-Sheng Ye. 2019. Migration of Rural Residents to Urban Areas Drives Grassland Vegetation Increase in China's Loess Plateau. Sustainability. 2019, 11 (23), 6764. DOI: 10.3390/su11236764. (IF2018=2.592)
8. Yue-Yuan Yu, Neil C. Turner, Yan-Hong Gong, Feng-Min Li, **Chao Fang**, Li-Juan Ge, Jian-Sheng Ye*. 2018. Benefits and limitations to straw- and plastic-film mulch on maize yield and water use efficiency: A meta-analysis across hydrothermal gradients. European Journal of Agronomy. 2018, 99, 138-147. DOI: 10.1016/j.eja.2018.07.005. (IF2018=3.384)
9. Jiansheng Ye*, Jiuying Pei, **Chao Fang**. 2018. Under which climate and soil conditions the plant productivity–precipitation relationship is linear or nonlinear? Science of the Total Environment. 2018, 616-617, 1174-1180. DOI: 10.1016/j.scitotenv.2017.10.203. (IF2018=5.589)
10. Ziqiang Yuan, Kailiang Yu, Xiaokang Guan, **Chao Fang**, Ming Li, Xiaoyan Li, Fengmin Li*. 2016. Medicago sativa improves soil carbon sequestration following revegetation of degraded arable land in a semi-arid environment on the Loess Plateau, China. Agriculture Ecosystems and Environment. 2016, 232, 93-100. DOI: 10.1016/j.agee.2016.07.024. (IF2018=3.954)

11. Ziqiang Yuan, Kailiang Yu, Howard Epstein, **Chao Fang**, Junting Li, Qianqian Liu, Xuewei Liu, Wenjuan Gao, Fengmin Li*. Effects of legume species introduction on vegetation and soil nutrient development on abandoned croplands in a semi-arid environment on the Loess Plateau, China. Science of the Total Environment. 2016, 541, 692-700. DOI: 10.1016/j.scitotenv.2015.09.108. (IF2018=5.589)
12. Chao Fang, Ziqiang Yuan, Xuewei Liu, Jiuying Pei, Dongmin Zhao, Jiansheng Ye*. 2015. Effects of slope on soil properties and the interaction of Caragana tibetica shrub species in Loess Plateau of China. Journal of Desert Research, 35(6), 1607-1611. (In Chinese with English Abstract)

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