ISSN (Online): 2349-1183; ISSN (Print): 2349-9265
TROPICAL PLANT RESEARCH
The Journal of the Society for Tropical Plant Research

7(3): 529–540, 2020
DOI: 10.22271/tpr.2020.v7.i3.065

Research article

Ensuring the effects of climate warming; the sensitivity of controlling factors on soil respiration in Sub-Tropical grassland

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Abstract: Prevailing climate change is expected due to carbon dioxide emission to the atmosphere through soil respiration and perhaps the alteration in the terrestrial carbon cycle. The measurements to establish the effect and sensitivity of soil temperature, soil water content and plant biomass on soil respiration was performed in the sub-tropical grassland located in Central Nepal. Field measurements of soil respiration was conducted by using the closed-chamber method, and soil temperature, soil water content and plant biomass were monitored in the years 2015 and 2016. The soil respiration showed positive significant exponential function which accounted for 74.6% (R^2=0.746, $p<0.05$) of its variation with the soil temperature. The temperature sensitivity of soil respiration, Q_{10} value obtained was 2.68. Similarly, soil respiration showed a positive significant exponential function that accounted for 37.2% (R^2=0.372, $p<0.05$) of its variation with the soil water content. Remarkable seasonal and monthly variations were observed in soil respiration, soil temperature and soil water content, and the plant biomass as well followed the seasonal trend in variation of the soil respiration. Average soil respiration during measurements period was observed 325.51 mg CO_2 m^{-2} h^{-1} and the annual soil respiration of the grassland in the years 2015 and 2016 was estimated 592.35 g C m^{-2} y^{-1}. The study confirmed that soil temperature is the most influential primary factor in controlling soil respiration along with the soil water content and plant biomass. This research indicates that through emissions under the increasing temperature and precipitation, in the changing climate, the sub-tropical grassland could be an additional source of carbon dioxide to the atmosphere that might spur risk for further warming.

Keywords: Sub-tropical ecosystem - Soil temperature - Soil water content - Temperature sensitivity - Plant biomass.

[Cite as: Dhital D, Prajapati S, Maharjan SR & Ohtsuka T (2020) Ensuring the effects of climate warming; the sensitivity of controlling factors on soil respiration in Sub-Tropical grassland. *Tropical Plant Research* 7(3): 529–540]

INTRODUCTION

The largest carbon pool is unexpectedly accumulated in terrestrial ecosystems and 2700 Gt of carbon is collected in the soil; this amount is much higher than the sum of the carbon reserve in the atmosphere (780 Gt) plus the plant biomass (575 Gt) (Lal 2008). When soil including plant roots and microorganisms respire and emit carbon dioxide (CO_2) as derived production during the biological process, it is referred as soil respiration. Soil respiration is varied by different biotic or abiotic factors that can cause a major impact on global carbon balance (Davidson & Janssens 2006). Soil respiration is the largest phenomenon of carbon exchange between

Received: 13 January 2020

https://doi.org/10.22271/tpr.2020.v7.i3.065

soil and atmosphere, even small fluctuation in soil respiration affects the global soil carbon significantly, and thus it plays an import role in the global carbon budget, (Luo & Zhou 2006). Small rise in terrestrial soil respiration results in higher accumulation of CO_2 in the atmosphere (Vargas *et al.* 2011); therefore the abiotic or environmental factors commonly temperature and soil moisture affect soil respiration considering responsible for the emission of carbon dioxide to the atmosphere (Chen *et al*. 2017, Yang *et al*. 2018).

Grassland comprises approximately 30% of the Earth's landmass and stores 28–37% of the terrestrial soil organic carbon (SOC) which is the largest among four major natural biomes (Lal 2004). Owing to the imbalance and higher records of carbon dioxide (CO_2) accumulation in the atmosphere, carbon exchange has become a major highlighted research topic for the ecologist around the world (Frank 2002, Kao & Chang 2009, Casanovas *et al*. 2012, Dhital *et al*. 2014, Bao *et al*. 2016, Yang *et al*. 2018). Most of the ecosystem carbon exchange researches are kept in priority to focus on forest habitat in the terrestrial ecosystem (Olajuyigbe *et al*. 2012, Du *et al*. 2013) and very less attention has been given to the grasslands (Adams *et al*. 1990, Sims & Risser 2000). However, studies suggest that soil respiration commonly is influenced by the variation in the existing vegetation type (Raich & Schlesinger 1992, Raich & Tufekcioglu 2001, Kao & Chang 2009, Tufekcioglu *et al*. 2009, Feng *et al*. 2017). Thus, the interpretation of the environmental factors affecting soil respiration in different types of vegetation might provide outright information for better understanding of local or global scale carbon cycle.

The most important link in the carbon cycle of the grassland ecosystem is grassland soil CO_2 emissions to the atmosphere through soil respiration (Tufekcioglu *et al*. 2001, Wang *et al*. 2016), and the amount of soil respiration directly determines the soil carbon turnover rate (Vesterdal *et al*. 2011). The extensive fibrous root system in grassland soils help to create an ideal environment for microbial activity that is why grassland soils contain high soil organic carbon (Conant *et al*. 2001). Both autotrophic or root respiration and heterotrophic or microbial respiration combines to form the soil respiration (Raich & Schlesinger 1992). Grasslands also behave as sink/source of atmospheric carbon but it depends upon the factors like climate, grazing intensity and land use management (Frank 2002, Smith 2014). Different studies suggested that small variation in carbon storage in grassland soil affects the concentration of atmospheric carbon due to its sensitivity, and hence affects the trend of local or global climate (Prentice *et al*. 2000, Schlesinger *et al*. 2000).

The amount of soil respiration that occurs in an ecosystem is directed by several factors, and among them, temperature, soil moisture, plant biomass, Photosynthetic Photon Flux Density (PPFD, light) and vegetation are the common factors that influence the imbalance of soil respiration (Cao *et al*. 2004, Davidson & Janssens 2006, Kim *et al*. 2010, Bao *et al*. 2016, Dhital *et al*. 2019). It has been well understood that environmental factors such as soil temperature and soil moisture are important abiotic parameters that are sensitive to the soil respiration and are commonly related to underlying processes in terrestrial (Davidson *et al*. 1998, Hashimoto *et al*. 2009, Kim *et al*. 2010, Wang *et al*. 2010, Guntiñas *et al*. 2013, Shen *et al*. 2015, Wang *et al*. 2016, Dhital *et al*. 2019).

Some studies of carbon exchange has been conducted in different ecosystems, such as forest, grassland, farmlands, etc around the world (Koizumi *et al*. 1999, Kim *et al*. 2010, Casanovas *et al*. 2012, Wang *et al*. 2016, Yang *et al*. 2018) but it is limited to the temperate grassland ecosystem in Nepal (Dhital *et al*. 2019) and not even yet observed in the tropical ecosystem. However, being aware of the consequences of prevalent changing climate from regional to the global scale, studies related to carbon sequestration in the forest ecosystem has been conducted since the past several years (Baral *et al*. 2009, Department of forest research and survey (DFRS 2015, Dhakal *et al*. 2017). The present study is aimed to research soil respiration dynamics with the measurements to understand the controlling factors such as soil temperature, soil moisture and plant biomass on soil respiration, and their impact on changing the climate in sub-tropical grassland ecosystem. This research assists in determining the sensitivity of these ecological parameters/factors of the ecosystem on the variation of carbon dioxide emission through soil respiration in different seasons and months of the year.

MATERIALS AND METHODS

Study area

The field study area was located in sub-tropical grassland, Katunje, Suryavinayak (27° 38′ 55.752″ N and 85° 25′ 16.248″ E; altitude of 1418 m a.s.l.), Bhaktapur Municipality of Central Nepal (Fig. 1). The grassland area is grazed by the domestic animals and it is surrounded by sub-tropical dense mixed forest protected by the local community which is the habitat of wild animals, birds, etc, and some cultivated land, roads, few settlements and non-forest lands in use. The region has sub-tropical monsoon climate with rainy summer (June–September) and dry winter (December–January). The grassland vegetation is dominated by *Paspalum* species and *Danthonia* species, and the other common species are *Drymaria cordata* (L.) Willd. ex Schult., *Ageratina* *adenophora* (Spreng.) R.M.King & H.Rob., *Eragrostis* species, *Thallictrum* species, *Osbeckia nepalensis* Hook., etc. (KATH, Herbarium).

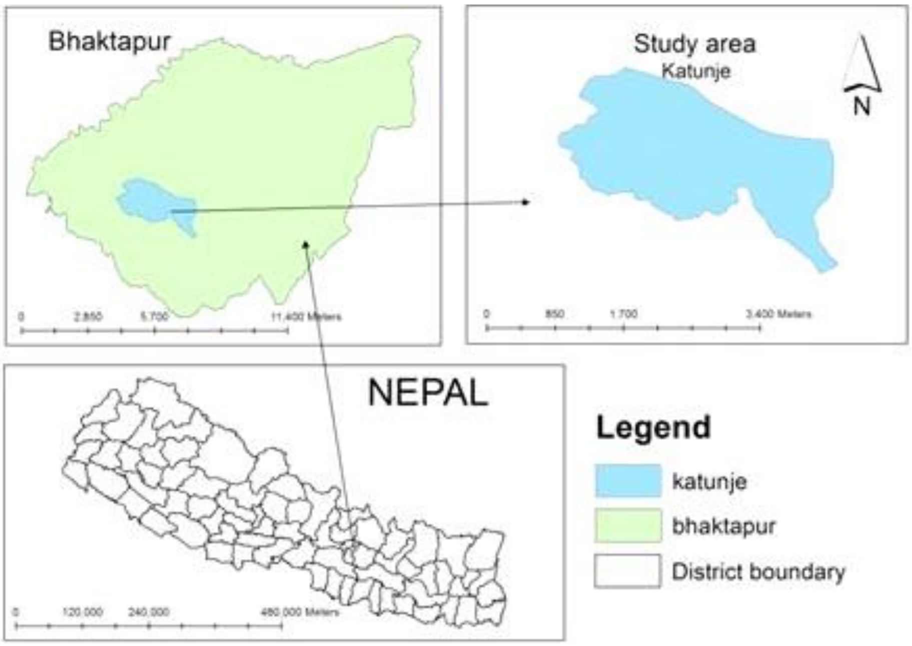

Figure 1. Map of Nepal, Bhaktapur Municipality and the study area (Katunje).

Soil respiration

The chambers (n=10) were randomly placed in the study area of size 50 m × 70 m and inserted 2 cm below the ground surface into the soil. The above-ground green plants at the surface inside the chamber were removed to separate the above-ground plant respiration from the soil. Soil respiration measurements were carried out one day after the chamber placement to eliminate the installation effect on soil respiration measurements. The measurements of soil respiration were carried out monthly from August 2015 to March 2016 between the 10:00 am and 2:00 pm. Vaisala CARBOCAP CO_2 probe GMP343 was used for the measurement of CO_2 concentration and gas temperature inside the chamber. This method involves placing a chamber over the soil surface and the increase in the concentration of CO_2 within the chamber is measured as a function of time by using closed chamber method with an infrared gas analyzer (IRGA). The chambers used for the measurements were cylindrical with 18 cm in diameter and 16 cm in height and made of polyvinyl chloride. Chambers were composed of two parts, a lid and a body. This lid was equipped with an IRGA for the measurement of CO_2 and gas temperature inside the chamber.

Environmental factors

Soil temperature and soil water content (SWC) at 5cm soil depth were recorded simultaneously at three points near each chamber during the measurement of soil respiration. The soil temperature was measured with a digital lab stem thermometer (AD-5622, A&D, Japan). Similarly, the soil water content was measured with TRIME-FM (Imko, Germany). Long term (2006 to 2017) meteorological data such as, the air temperature and precipitation of the study area were generated from the Department of Hydrology and Meteorology (DHM), Nepal. Continuous measurement (1 h interval) of the soil temperature at 5 cm soil depth was also recorded by Onset TidbiT v2 temperature data logger near (100 m apart from the study site) the grassland area.

Plant biomass (above- and below-ground)

Above-ground plant biomass and below-ground root biomass was measured at five random plots within the study area. The above-ground plant parts were cut at the ground level within the quadrat of size 20 cm × 20 cm. The soil sample below 15 cm depth was also collected inside the quadrat of size 20 cm × 10 cm for the measurement of root biomass. The roots from the soil were manually separated, sieved and washed properly to remove all associated soil in the root. Both above- and below-ground plant parts were then dried at 70°C for 48 h, and weighed with an electronic balance. The dry weights of the biomasses were then calculated.

Calculation of soil respiration and data analysis

The soil respiration was calculated from equation (1) (Koizumi *et al*. 1999) as follows:

$$F = (V/A)\ (\Delta c/\Delta t) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Where, F is the soil respiration (mg CO_2 m^{-2} h^{-1}), V is the volume of air within the chamber (m^3), A is the area of the soil surface within the chamber (m^2), Δc & Δt is the time rate of change of CO_2 concentration in the air within the chamber (mg CO_2 m^{-3} h^{-1}).

When the CO_2 concentration is plotted against time, relationships of linear regression can be ascertained (Bekku *et al*. 1995, Koizumi *et al*. 1999). The $\Delta c/\Delta t$ is calculated using this linear regression coefficient.

To estimate soil respiration to soil temperature, an equation of exponential regression (Bao *et al*. 2010, Dhital *et al*. 2010a, Meyer *et al*. 2018) was used which are as follows:

$$F\,(T) = a \times \exp\,(b \times T) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where, $[F(T)]$ is the estimated soil carbon emission rate (mg CO_2 m^{-2} h^{-1}) at soil temperature (T°C) at 5 cm soil depth, a represents the intercept of soil respiration rate when ST is zero, and b represents the temperature sensitivity of soil respiration.

The b value was used to calculate a coefficient of temperature sensitivity (respiration quotient, Q_{10}), which describes the change in soil carbon emission over a 10°C increase in soil temperature by equation (3).

$$Q_{10} = \exp\,(b \times 10) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Annual soil respiration and the missing values of soil respiration for the month of April, May, June and July was calculated by using the continuous measurements data of the soil temperature at 5 cm soil depth of the study area and the above equation (2) generated from the relationship between the soil respiration and soil temperature of the measurements.

The biomasses of the above-ground and below-ground plant parts were calculated by using the following formula:

$$\text{Biomass} = \text{Dry weight (g)} / \text{Area } (m^2)$$

RESULTS

Micro-meteorological data

The annual mean (2006–2017) air temperature and precipitation of the study area were recorded 19.6°C and 124.07 mm, respectively (DHM 2016). And maximum mean air temperature was 24.9°C in June during the summer season (June to August) and minimum was 11.5°C in January during the winter season (December to February) (Table 1). Similarly, maximum mean precipitation was recorded 375.20 mm in July and the minimum was 1.9 mm in November (Fig. 2). The maximum and minimum soil temperature of the study area was 22.7°C in June and 9.7°C in December, respectively which was obtained from the temperature data logger in continuous measurements (Fig. 3).

Table 1. Maximum and minimum air temperature (2006-2015), soil temperature at 5 cm soil depth, above-ground biomass, below-ground biomass and soil respiration of the study area.

	Air Temperature (°C)	**Soil Temperature (°C)**	**Soil Water Content (%)**	**Above-ground biomass (g d w m^{-2})**	**Below-ground biomass (g d w m^{-2})**	**Soil Respiration (mg CO_2 m^{-2} h^{-1})**
Maximum	25.7#	29.2	47.88	1428	917.5	1055.33
Minimum	10.1#	7.3	0.79	71.5	46.5	45.38
Average Maximum	24.93**	28.17*	43.04*	778.4*	624.6*	739.68*
Average Minimum	11.51**	7.83*	9.68*	172.65*	195.95*	90.71*

Note: #Monthly mean; *Mean n= 10; **Average 2006–2017.

Temperature effect on soil respiration

The soil respiration was represented by the positive exponential function of soil temperature which was determined by the measurements of soil respiration with the increase in the concentration of carbon dioxide within the chamber as a function of time (Eq. 1, 2). The soil respiration was accounted by 74.6% variability of the soil temperature and that was statistically significant ($p<0.05$) (Fig. 4). The temperature sensitivity of soil respiration *i.e.* variation in soil respiration in each 10°C increase in soil temperature at 5cm soil depth, Q_{10} value in the study was 2.68.

Seasonal and monthly variation of soil respiration and soil temperature at 5 cm soil depth was observed throughout the year (Fig. 5). The highest soil respiration measured in the grassland was 739.67 mg CO_2 m^{-2} h^{-1} at the time of maximum soil temperature at 28.17°C in September. The lowest soil respiration measured was 90.71 mg CO_2 m^{-2} h^{-1} in February at low soil temperature at 10.05°C, but the lowest soil temperature was recorded at 7.83°C in December (Table 1). The similar comparative trend of variation of the soil respiration and soil temperature was observed throughout the measurements.

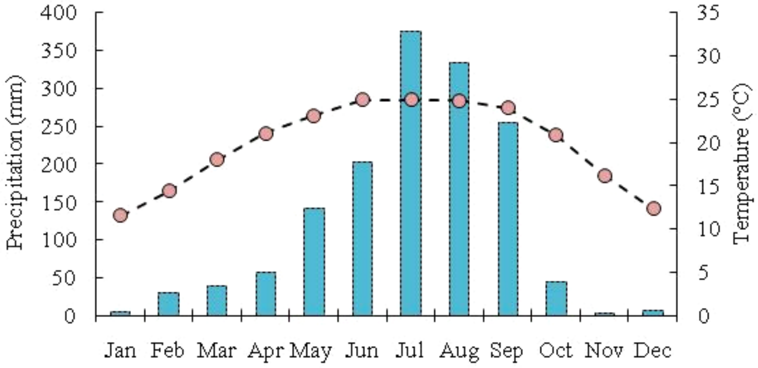

Figure 2. Monthly mean precipitation and air temperature of the study area from 2006 to 2017 (Source: Department of hydrology and meteorology (DHM), Kathmandu). Bar, precipitation; Filled circle, air temperature.

Figure 3. Daily average soil temperature (°C) at 5 cm soil depth continuously measured (one hour interval) in the study area (100 m apart from study site, from August 2015–July2016).

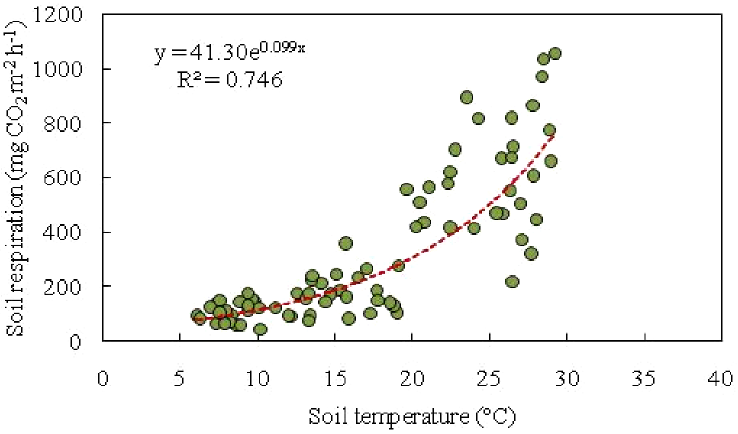

Figure 4. Relationship between soil respiration (mg CO_2 m^{-2} h^{-1}) and soil temperature (°C) at 5 cm soil depth.

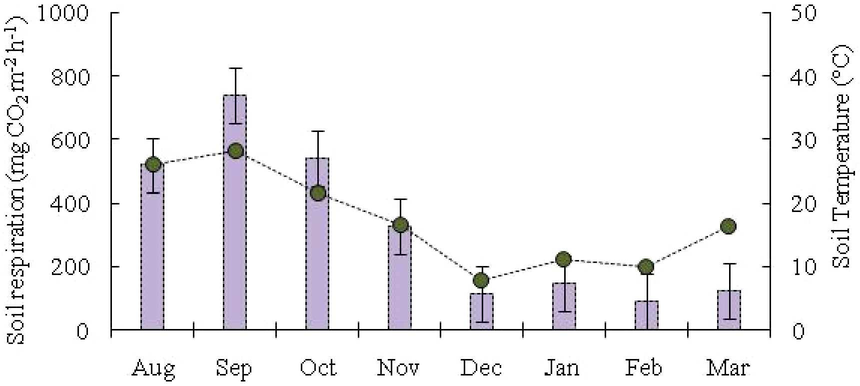

Figure 5. Seasonal and monthly variation of soil respiration and soil temperature at 5 cm soil depth (August 2015 to March 2016), n= 10. Bar, soil respiration; Filled circle, soil temperature.

Soil water content effect on soil respiration

The effect of soil water content on soil respiration of the grassland was analyzed with the relation of the positive exponential function (R^2=0.372) (Fig. 6). The soil respiration was accounted by 37.2% variability of the soil water content and it was statistically significant ($p<0.05$). In the study, higher soil respiration values were obtained between 25% and 45% of the soil water content throughout the measurement. The soil water content ranged from 0.79% (lowest) in January to 47.88% (highest) in August. The maximum monthly average (n=10) soil water content value recorded was 43.05% in August and the minimum recorded was 9.68% in March (Table 1).

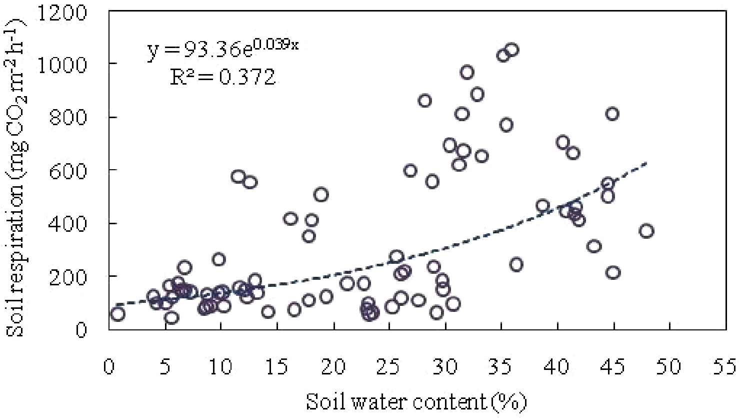

Figure 6. Relationship between soil respiration (mg CO_2 m^{-2} h^{-1}) and soil water content (%) at 5 cm soil depth.

Seasonal and monthly variations of soil respiration and soil water content were visible (Fig. 7). Soil Water Content (SWC) decreased gradually from August to January while there was a slight increase in SWC in February. The highest value of monthly average (n=10) soil water content was observed in August at the time of slightly lower (comparatively lower than September) soil respiration (518.78 mg CO_2 m^{-2} h^{-1}). However, both soil respiration and soil water content showed compatible decreasing tend from the wet summer season to the dry winter season.

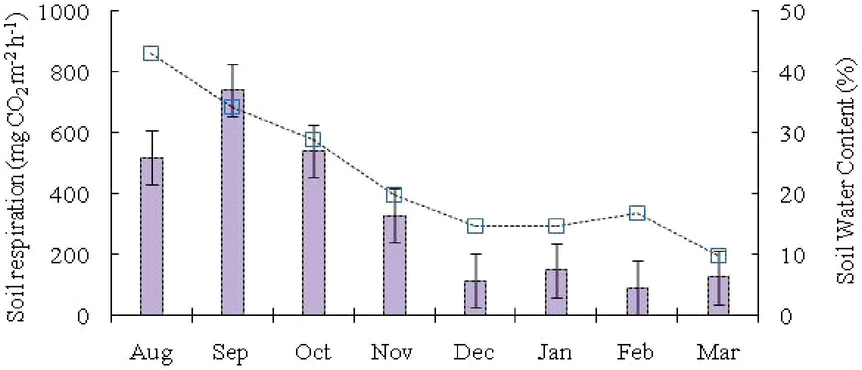

Figure 7. Seasonal and monthly variation of soil respiration and soil water content at 5 cm soil depth between (August 2015 to March 2016), n=10. Bar, soil respiration; Open square, soil water content.

Plant biomass effect on soil respiration

The above-ground plant biomass ranged from 778.4 g d w m^{-2} (n=10) in November to 172.65 g d w m^{-2} (n=10) in March and the highest value was obtained at 1428.0 g d w m^{-2} in January and the lowest at 71.7 g d w m^{-2} was in October (Table 1). The seasonal and monthly variations of the above-ground biomass and soil respiration were visible but not compatible to each other as the above-ground plant biomasses were higher during the period of lower soil respiration in winter. A slightly negative correlation was observed between soil respiration and above-ground plant biomass ($r=-0.049$, $p>0.05$) which was statistically insignificant (Fig. 8a).

The below-ground plant biomass showed comparable and similar decreasing trend of the soil respiration from the growing summer season to the dry winter season. Higher below-ground root biomass was obtained during plant growing season and lower during the winter season. The below-ground root biomass showed a moderate positive correlation ($r=0.46$, $p>0.05$) with soil respiration, but the relationship was statistically insignificant (Fig. 8b). The highest value of below-ground plant biomass was obtained in August (917.50 g d w m^{-2}) and the lowest value (46.50 g d w m^{-2}) was obtained in September. The average (n=10) maximum below-ground root biomass was 624.6 g d w m^{-2} in August and the minimum was 195.95 g d w m^{-2} in January (Table 1).

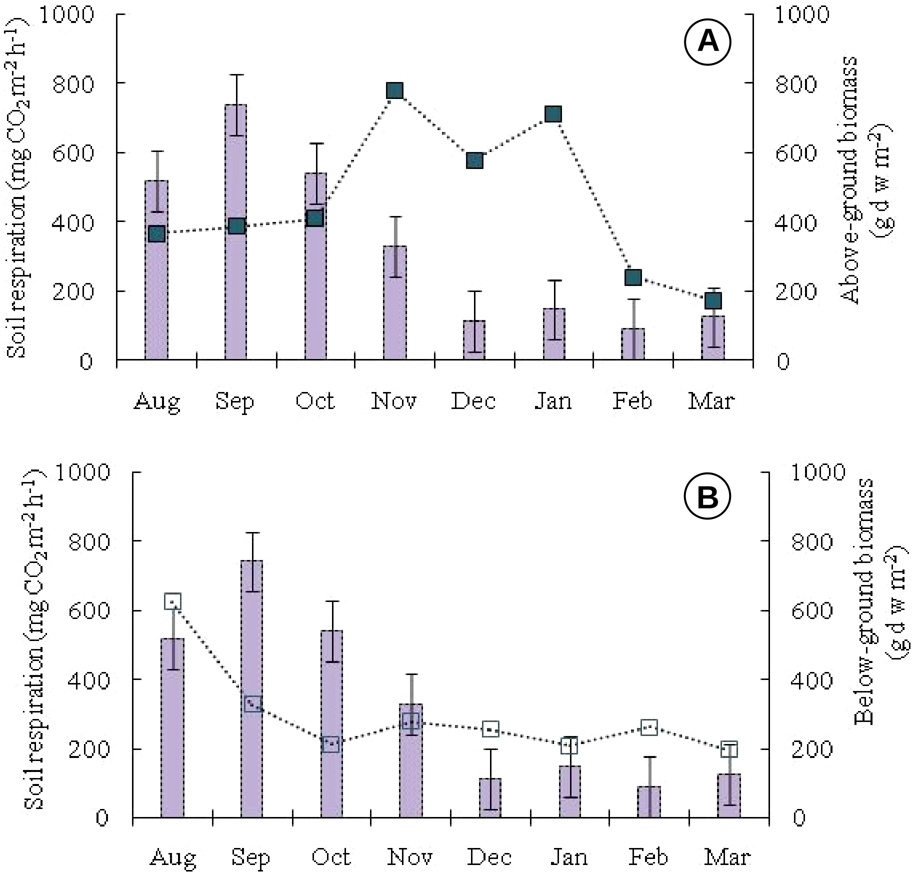

Figure 8. Monthly variation of soil respiration and plant biomass (August 2015 to March 2016): **A**, Above-ground; **B**, Below-ground. [Bar, soil respiration; Filled square, above-ground biomass; Open square, below-ground biomass]

Annual variation of soil respiration

The mean soil respiration throughout the measurement period (August–March) of the grassland was 325.51 mg CO_2 m^{-2} h^{-1}. The soil respiration (average n=10) increased in September (739.68 mg CO_2 m^{-2} h^{-1}) from August (518.78 mg CO_2 m^{-2} h^{-1}) with increasing soil temperature. The lower values of soil respiration with the lower soil temperatures were observed during the winter season (December–February). The soil respiration started increasing as soil temperature began to rise from March to July. The rate of soil respiration remained higher in August, September and October (Fig. 9). The maximum and minimum soil respiration (average n=10) were 739.67 mg CO_2 m^{-2} h^{-1} (highest value 1055.33 mg CO_2 m^{-2} h^{-1}) and 90.71 mg CO_2 m^{-2} h^{-1} (lowest value 45.38 mg CO_2 m^{-2} h^{-1}) in September and February, respectively (Table 1). The annual soil respiration of the grassland estimated (Eq. 2) during the study period in the year of 2015/2016 was 592.35 g C m^{-2} y^{-1}.

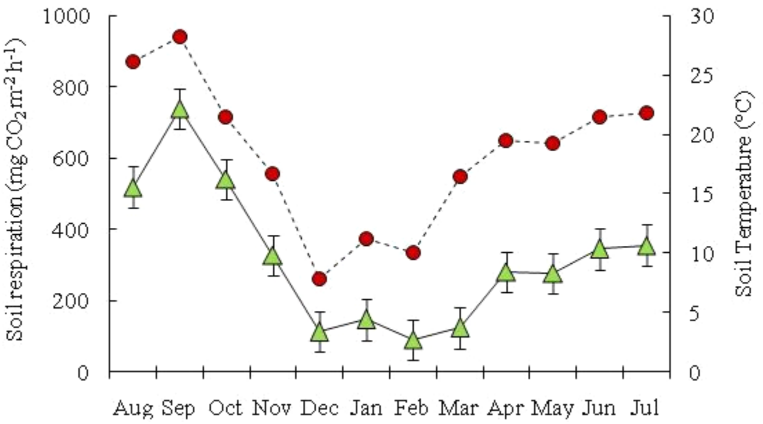

Figure 9. Annual-monthly variations of soil respiration and soil temperature (August 2015 to July 2016). Filled triangle, soil respiration; Filled circle, soil temperature.

DISCUSSION

Temperature is regarded as the most important influencing factor of the soil respiration variations, because soil temperature is the common driver of all the processes of biological activities in the soil as it affects the respiratory enzymes of both roots and soil microbial biomass (Xu *et al*. 2011). The changes in soil temperature accounted for 74.6% variation of the soil respiration in the study (Fig. 4), which is evident that the soil temperature is the primary factor influencing soil respiration of the sub-tropical grassland. Remarkable seasonal and monthly variations of the soil respiration and the soil temperature was observed in the study (Fig. 5), which have been investigated in some researches to determine the relationship between soil respiration rate and the soil temperature, but the variation and differentiation of its effects differs on ecological, functional, spatial, seasonal and vegetation traits (Cao *et al*. 2004, Dhital *et al*. 2010b, Inoue & Koizumi 2012, Bao *et al*. 2016, Li *et al*. 2017). The positive exponential higher (Fig. 4, R^2=0.746) relationship perceived between the soil respiration and soil temperature of the study is quite compatible with the research in other grassland ecosystems (Kucera & Kirkham 1971, Raich & Schlesinger 1992, Raich & Potter 1995, Mielnick & Dugas 2000, Dhital *et al*. 2010a, Inoue & Koizumi 2012, Dhital *et al*. 2019). The pronounced soil temperature effect (R^2=0.746) on soil respiration in the study was remarked because of the variations of soil temperature from the lowest to the highest values obtained in different seasons and months of the year. The temperature sensitivity of soil respiration, Q_{10} value (2.68) was better found comparable (2.1–2.7) to the perennial warm season *Zoysia japonica* Steud. grassland in Japan (Dhital *et al*. 2010a) and the mean Q_{10} value (2.60) was observed in the studies of various Temperate to Tropical Chinese grasslands (Feng *et al*. 2018). However, the values of Q_{10} are variable and could not be fixed, to accurately define the ecosystem, vegetation and to quote the soil respiration in seasonal and inter-annual basis.

The sensitivity of the soil respiration for its variations in grassland is not limited to the soil temperature, but factor like, soil water content also plays an important role (Inoue & Koizumi 2012, Bao *et al*. 2016). In the present study, the soil respiration was responsive to the soil water content. The positive significant exponential function (R^2 = 0.372) between soil respiration and soil water content showed that the incline in soil respiration caused by the increase in soil water content (Fig. 6). This effect on soil respiration and carbon dioxide evolution by soil water content has also been reported in some previous researches (Johnson *et al*. 1994, Qi *et al*. 1994, Guntiñas *et al*. 2013, Shen *et al*. 2015, Li *et al*. 2017, Dhital *et al*. 2019). However, Bao *et al*. (2016) in a review of studies analyzed the effect of soil temperature and moisture on soil respiration of Tibetan plateau which resulted that the soil moisture alone had no effect on soil respiration suggesting that soil moisture is not limiting factor on soil respiration at a lower temperature. Dhital *et al*. (2019) in Temperate grassland observed the negative exponential effect of soil water content on soil respiration and explained the soil respiration could be decreased when the soil water content crossed its higher limit (above 35%). Similar, decreasing trend of the soil respiration with the decrease in soil water content have been identified in the seasonal and monthly variations of the soil respiration and soil water content of the study (Fig. 7). Similarly in a separate study, only the positive linear relationship was observed in summer season and no significant effect of soil water content on soil respiration was observed in spring and autumn season in a Temperate grassland that was due to high soil water content and high soil temperature in summer (Inoue & Koizumi 2012).

In the present study, the level of soil water content could not reach its optimum limit to overcome the soil respiration, and the relationship between soil respiration and soil water content was positive exponential function (R^2 = 0.372) just enough to well define the effect of soil water content on soil respiration. Earlier studies also mentioned about the functional relationships between soil respiration and soil water content, and the effect of both factors; soil temperature and soil water content, on soil respiration (Lee *et al*. 2002, Li *et al*. 2008, Marcolla *et al*. 2011, Guntiñas *et al*. 2013, Dhital *et al*. 2014, Jeong *et al*. 2018). However, the research showed that with limited precipitation, microbes and plant roots have to invest more energy to produce protective elements which would inhibit both growth and respiration (Li *et al*. 2017).

The above-ground plant biomass was followed the common seasonal trend of the soil respiration, that was not directly visible in the study and couldn’t obtained the values of higher soil respiration at the time of higher plant biomass during the plant growing season. The higher soil respiration values were observed at the time of lower above-ground plant biomass (August–October) and lower soil respiration was obtained at the time of high above-ground plant biomass in December that was owing to the intensive grazing during the months of the plant’s growing season when the soil respiration was increased with the increasing rate of temperature and precipitation in summer, and the grazing was not accounted during the biomass measurements in the study. The above-ground plant biomass was observed higher during winter season because of the plant biomass remaining from the growing season and very little grazing activities during winter season. Thus, relationship between soil respiration and above-ground plant biomass could be assumed, and but invisible that might be observed when the measurements are made in exclusory fenced areas.

The below-ground root biomass showed a seasonal trend of soil respiration, where higher below-ground root biomass was obtained during plant growing season (August–September) and it was followed by decreasing rate of root biomass during non-growing season in winter (December–March), and the soil respiration was also observed accordingly in the study period (Fig. 8b). The research conducted in the grassland of Tibetan plateau reported a significant positive correlation between soil respiration and root biomass, where root biomass accounted 30–64% of soil respiration variation (Bao *et al*. 2016), which is comparable to the seasonal variation of the soil respiration and below-ground root biomass observed during the study (Fig. 8b). However, Geng *et al*. (2012) found that only below-ground biomass and soil moisture had direct effects on soil respiration. While other factors either indirectly or directly affects the soil respiration through the below-ground biomass and effects on net ecosystem production (Tufekcioglu *et al*. 2001, Dhital *et al*. 2010b). The maximum above-ground plant biomass of our study (Table 1) was comparable to the total above-ground plant biomass while reached its peak (820.0 g d w m^{-2}) in Temperate *Zoysia japonica* grassland of Japan (Dhital *et al*. 2010a), but, the maximum below-ground root biomass was comparatively lower in our study (Table 1). The above-ground and below-ground properties of the soil responded to the vegetation type that is likely to be influenced by the climatic variables, and the different plants differ in their response to variation in climatic factors and elevation (Sundqvist *et al*. 2011).

The finding of annual-seasonal variations of soil respiration in this sub-tropical grassland presents the higher emission in respiration during plant growing season which was peaked in September and then August with the higher soil temperature (Fig. 9). The soil respiration was decreased with decreasing soil temperature towards the end of the growing season and lowest soil respiration was reported during the months of winter season (December–February) and early spring (April). These reports comply to the findings of the warm season perennial Temperate grassland in Japan where lower soil respiration was observed from January to April and the maximum (1414 mg CO_2 m^{-2} h^{-1}) soil respiration was observed in August during the plant growing season (Dhital *et al*. 2014). The annual soil respiration of the present study (592.35 g C m^{-2} y^{-1}) was comparatively lower than the temperate *Zoysia* grassland (1,121.4 g C m^{-2} y^{-1}) in Japan (Dhital *et al*. 2010a), nearly equal to the mean annual soil respiration (582.0±57.9 g C m^{-2} yr^{-1}) and between the annual soil respiration of the temperate and warm tropical grasslands of China (Feng *et al*. 2018). The soil respiration of the grasslands significantly differed from each other which is determined by the grassland type referring the geographical locations, vegetations, altitudinal and climatic variations, the solar radiation, that is positively correlated with the mean annual temperature, precipitation, soil moisture, soil organic carbon content and aboveground biomass (Feng *et al*. 2018).

CONCLUSION

In sub-tropical grassland, taking into account the effect and sensitivity of environmental factors such as soil temperature and soil moisture, and the plant biomass, the measurement of soil respiration by using the chamber method showed the importance of these factors/parameters. And their variations are much more responsible to regulate the soil carbon dioxide emission in the grassland ecosystem. The significant positive exponential relationship was observed between soil respiration and soil temperature ($R^2=0.746$, $p<0.05$), and the soil respiration and soil water ($R^2=0.372$, $p<0.05$). Both soil temperature and soil water content was followed the seasonal trend of monthly soil respiration. Above- and below-ground plant biomass varied with the seasonal variations which were higher during plant growing summer season (grazing not accounted) and lower in the non-growing winter season. The annual soil respiration of the grassland was estimated at 592.35 g C m^{-2} y^{-1}. Findings confirmed that the soil temperature is the most confidential influencing factors in controlling soil respiration along with the soil water content and plant biomass. The results imply that the sub-tropical grassland could be the additional source of atmospheric carbon dioxide under increasing temperature and precipitation in changing climate and that together might be the risk of further warming.

ACKNOWLEDGEMENTS

We are thankful to the Nepal Academy of Science and Technology (NAST) for providing the equipments and necessary support throughout the entire research. We also express our thanks to the people of the local community in Katunje, Bhaktapur for their continuous co-operation and kind support during the study.

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