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Soil Carbon Dioxide Emission: Soil Respiration Measurement in Temperate Grassland, Nepal

Deepa Dhital[1*], Suman Prajapati[2,3], Sanu Raja Maharjan[2], Hiroshi Koizumi[4]

[1]Nepal Academy of Science and Technology (NAST), Khumaltar, Nepal
[2]Department of Environmental Science, Khwopa College, Dekocha, Nepal
[3]Sanima Hydro and Engineering Pvt. Ltd., Dhumbarahi, Nepal
[4]Faculty of Education and Integrated Arts and Sciences, Waseda University, Shinjuku, Japan
Email: *dhital.deepa@gmail.com, *deepa.dhital@nast.gov.np, prajapatisuman0609@gmail.com, botanysanu@hotmail.com, hkoizumi@waseda.jp

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Abstract

Soil carbon dioxide emission: soil respiration is representing a major contributor of accumulating carbon dioxide in the atmosphere that aids to accelerate global warming and altering the climate. Soil temperature, soil water content, sun light and vegetation are considered most common regulators of soil respiration variations in ecosystem. The soil respiration was measured in grassland intended to examine how the soil respiration changed with varying climatic factors, for two years (2015 and 2016) in temperate grassland of Annapurna Conservation Area (ACA), Nepal. In the study, soil temperature accounted exponential function of soil respiration variation at 42.9%, 19.1% and 23.3%, and temperature sensitivity of the soil respiration (Q_{10}) was obtained at 6.2, 1.4 and 1.8 in October 2015 and April 2016 and both the measurements were combined, respectively. Significant negative ($R^2 = 0.50$, $p < 0.05$, October 2015) and positive ($R^2 = 0.084$, $p < 0.05$, April 2016) exponential function of soil respiration and soil water content were determined, where high soil respiration values were always measured between 30% and 35% of the soil water content. However, linear significant relationship was determined ($R^2 = 0.376$, $p < 0.05$) between soil respiration and photosynthetic photon flux density (PPFD). Soil respiration value averaged in October 2015 was 357 mg CO_2 m^{-2} h^{-1} and in April 2016 it was 444.6 mg CO_2 m^{-2} h^{-1}. Above- and below-ground plant biomasses were obtained at 231.1 g d w m^{-2} and 1538.8 g d w m^{-2} in October, and at 449.9 g d w m^{-2} and 349.0 g d w m^{-2} in April, respectively. This study showed variation of soil respiration in relation to the factors such as soil temperature, soil water content and photosynthetic photon flux density signifying their importance in governing ecosystem function and carbon balance of the temperate grassland ecosystem.

Keywords

Soil Respiration, Soil Temperature, Soil Water Content, Photosynthetic Photon Flux Density, Temperate Grassland

1. Introduction

Soil is a good resource to human kinds as it is dynamic to accumulate more carbon than atmosphere and vegetation [1] [2]. However, multiple factors in association with soil degradation result in significant soil carbon losses [3] that affect overall carbon dynamics [4]. The efforts of soil restoration help prevent soil degradation with increase in soil organic carbon and mitigate climate change through sequestration [5] [6] [7] [8]. Soil carbon storage provides favorable conditions for increasing net primary productivity growth in grassland [9] [10]. Soil carbon storage helps reduce soil erosion through a permanent soil cover with dense rooting systems and carbon input [11], coupling elemental cycles [12] [13] and overall carbon balance as sink/source capacity of the ecosystem [14] [15].

The level of carbon in the atmosphere has increased exponentially (275 - 285 ppm [16] to 400 ppm [17]) and this continuous process is expected to go even higher (490 - 1370 ppm) by 2100 depending on representative concentration pathways [18]. The majority of additional atmospheric carbon dioxide comes naturally from soil through soil respiration. This phenomenon is a combined metabolic activity of roots and microorganisms generated mainly from soil surface by respiration and decomposition, respectively and it is considered the largest component of carbon cycling in grassland ecosystems [19] [20] [21] [22]. The soil carbon dioxide emission in respiration, as much as 50% - 90% of the annual gross primary productivity returns back to the atmosphere [23] depending on biotic and abiotic factors [24] [25] [26] [27] [28]. Thus, magnitude of soil respiration can turn the carbon budget from a net sink into a net source in altered environmental conditions of increased temperature and drought [29] [30] [31] [32]. However, the soil respiration response to these drivers varies among ecosystems, forest, grassland and tundra [22], and eco-regions [33].

The evidence shows that a small increase in terrestrial soil respiration can result in higher atmospheric carbon dioxide (CO_2) concentration, and ultimately feedbacks to global climate change [29] [34] [35]. Soil respiration can be measured and estimated which provides an evidence for the ecosystem carbon balance [14], and the carbon balance has become one of the most important ongoing issues in global carbon cycle [35] [36]. Two ecosystem processes mainly plant photosynthesis and soil respiration determines the balance of carbon input and storage, and the storage contributes more than the plants and atmosphere [2] [5]. The emission of carbon dioxide has high potential to increase and decrease the amount of atmospheric CO_2 that could feed back sequester carbon, and significantly exacerbate in mitigating climate change [8] [37] [38] [39]. However, soil carbon pool dynamics can be altered with the factors like climate change [40], land use [41] [42] [43] and management practices [21] [44] [45] [46]. Research indicated that global soil carbon dioxide emissions are in the range of 98 ± 12 $Pg·y^{-1}$ (1 Pg = 10^{15} g), with annual increases of 0.1 Pg that is suggested to be temperature-associated [47]. The storage of carbon in soil depends upon the carbon inputs, rate of decomposition of soil organic matter, soil texture and climate [48].

Multiple researches proved that the temperature, soil moisture, light intensity and plant growth affects on soil respiration, and the measurement of the soil respiration were conducted to reveal the effect of these factors to the variation of soil respiration, and overall change in the climate [20] [21] [32] [49] [50]. Scientific studies in terrestrial ecosystem have indicated that the temperature is directly interrelated to the warming climate of global change, which has high response to the variations of soil respiration *i.e.* to the rising label of atmospheric carbon [20] [23] [33] [36] [51]. The precipitation, soil water content, light intensity and vegetation growth have very common effect on soil respiration variations, and increasing soil respiration that ultimately depends on variability of measurement time/length and the existing climate during measurement period [22].

Geographical distribution of grassland is expansive throughout the world [38] and it is one of the principal ecotypes in the terrestrial ecosystem situated mostly in areas with more severe eco-environment where neither the forest growth nor the farmland reclamation is appropriate. Carbon in grasslands originates from below-ground biomass [14] [21] [52], primarily roots [53] [54] that increase with the age [55] and micro-organisms [43]. Approximately above 40% land area of the global terrestrial ice-free surface is covered by grasslands [56]. Globally, the grassland varies from sub-tropical, tropical and temperate region to the alpine meadow with variation of altitudinal gradient from North to the South. The temperate regions are the common eco-regions of Asia and are most common in Nepal, which occupies 20% to 29% of the total land area, covered by the grassland and is distributed from east to the larger area in the west (Source: [57]).

Very few researches have been focused in the study of soil carbon dioxide emission *i.e.* soil respiration and these researches are not sufficient to well explain the effect of environmental factors on soil respiration, in the temperate region grassland ecosystem. Temperate regions are kept as most prioritized regions considering its vulnerability due to increasing level of atmospheric carbon dioxide, continuous warming atmosphere and climatic change. Thus, the present study was conducted with the aim to measure the soil respiration in a temperate grassland ecosystem which is located in Annapurna Conservation Area (ACA), Nepal. The study further aimed to evaluate the relationship between soil respiration and different environmental factors like soil temperature, soil water content, photosynthetic photon flux density (PPFD), and plant biomasses.

2. Materials and Methods

2.1. Study Site

The study was conducted in grassland located at 2160 m a.s.l. in Annapurna Conservation Area of west central region (N 28˚22'23.7", E 083˚48'18.0") in Nepal (**Figure 1**). The grassland covers an area of 10,872 m^2. The Annapurna Conservation Area is a largest protected area of Nepal covering 7629 km^2 which is situated at the Annapurna range of Himalayas across Manang, Mustang, Kaski, Myagdi and Lamjung districts. The area ranges in altitude from 790 m a.s.l. to the peak of Annapurna I at 8091 m a.s.l. Climate of the study area is warm temperate with much less rainfall in winter than the summer. The annual mean temperature and rainfall from 2005 to 2014 were 16.5˚C and 441.21 mm, respectively (recorded at Lumle, Department of Hydrology and Meteorology, DHM 2016). The region has higher rainfall than the eastern region, and most rainfall occurred in July and August, whereas less rainfall and in some years no rainfall events occurred from November to December. The temperatures were recorded highest from July to August and lowest from January to December (DHM).

The study area was primarily dominated by *Digitaria* species (e.g. *Digitaria ischaemum*, *Digitaria sanguinalis* and *Digitaria flaccida*) and *Potentilla fulgens* but *Centella asiatica*, *Geranium* species and *Anaphalis margaritaceae* were also common. The grassland area was surrounded by a dense *Daphniphyllum himalense* forest which was the habitat of wild animals, birds, etc and grazed with domestic and wild herbivores.

2.2. Environmental Factors

Air temperature and precipitation from 2005 to 2014 of the study area were received from the records available at the Department of Hydrology and Meteorology (DHM), Nepal. Soil temperatures and soil water content at 5 cm soil depth were measured at three different points near the chamber, during each soil respiration measurement. 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). Photosynthetic Photon Flux Density (PPFD, light) was measured by using a data logger with LI-190SA quantum sensor (LI-COR Inc.) placed on top of the chamber at three points during each soil respiration measurement. The PPFD was measured in October 2015 and the measurement was not possible to make in April 2016 due to some technical glitch.

2.3. Soil Respiration

Soil respiration measurements were conducted in an area of size 50 m × 70 m within the study site. Closed chamber method with infrared gas analyzer (IRGA) technique was used for the measurement of soil respiration. The cylindrical chambers ($n = 10$) made of polyvinyl chloride of size 18 cm diameter and 16 cm height ($n = 10$) were used for the present study were composed of two parts, a lid

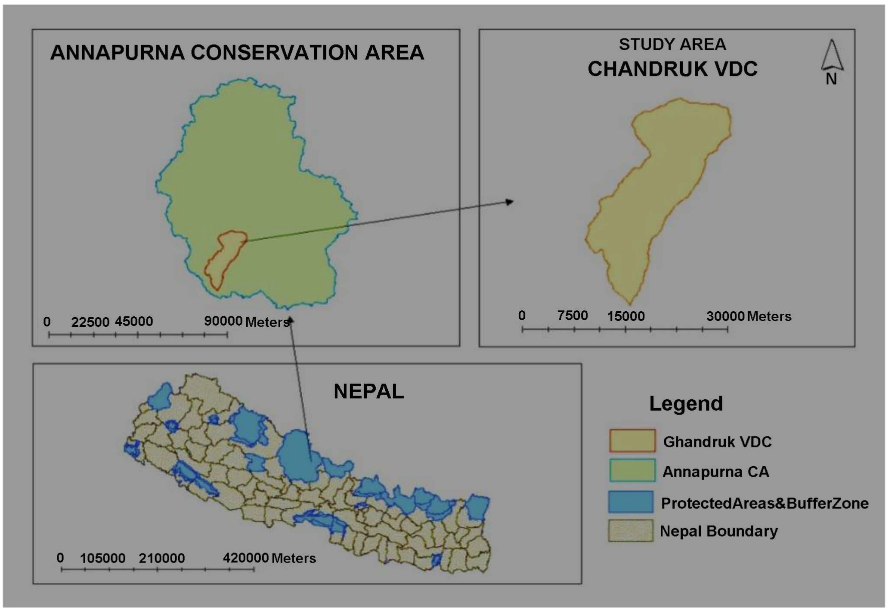

Figure 1. Map of Nepal, Annapurna Conservation Area and study area (Ghandruk).

and a body. This lid was equipped with an IRGA for the measurement of CO_2 and gas temperature of the chamber (body). 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 increase in concentration of CO_2 within the chamber is measured as a function of time and logger (VAISALA humicap hand held device) was used to record the measured data. The chambers were randomly placed in the study area and they were inserted at 2 cm soil depth one day prior to the soil respiration measurements in order to avoid the installation effect and prevent instability of data records during the measurements. All living vegetations above the soil surface inside the chamber were clipped at the time of chamber installation to avoid above-ground plant respiration. Soil respiration was measured in the morning between 7:00 am and 9:00 am, afternoon between 11:00 am and 1:00 pm and evening between 3:00 am and 5:00 pm on 27th, 28th and 29th in October 2015 and 19th, 20th and 21st in April 2016. Three replications of soil respiration measurements were made in each chamber. All measurements were conducted at 2 hours of time intervals within a day, on different dates and seasons in order to relate the soil respiration with varying temperature, soil moisture and light, and plant biomasses, and observe the effect of these factors on soil respiration.

2.4. Plant Biomass

Above-ground plant biomass and below-ground root biomass were measured at five random plots within the study area in the course of measurements of soil respiration in October 2015 and April 2016. Above-ground plant parts were clipped at ground level within five sample quadrats each of size 20 cm × 20 cm. The below-ground root biomass was sampled within five sample quadrats of each size 20 cm × 10 cm. The root samples were extracted up to 15 cm soil depths. The roots from the soil were manually separated, sieved and washed properly in order to remove all associated soil in the root. Both above-ground and below-ground parts were then oven dried at 70˚C for 48 h, and weighed with an electronic balance. The dry weights of the biomasses were then calculated.

2.5. Data Analysis

Calculation of soil respiration was made from Equation (1) [58] as follows:

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

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 the 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 [58] [59]. The $\Delta c/\Delta t$ is calculated using this linear regression coefficient.

The soil respiration was estimated with the relation of soil temperature; an equation of exponential regression [14] [24] [33], which were used as follows:

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

where $F(T)$ is the estimated soil respiration 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.

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 respiration over a 10˚C increase in soil temperature by Equation (3).

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

Soil samples for calculating bulk density of the soil were extracted using a soil core sampler. Soil samples (n = 5) were oven dried at 80˚C for 48 h and it was calculated dividing dried soil by volume of the core.

3. Results

3.1. Environmental Factors

Monthly ten years mean (2005 to 2014) air temperature and precipitation in the study area showed that temperature started to increase from May with the initiation of plant growing season (Department of Hydrology and Meteorology, DHM at Lumle). In January and December the mean (2005 to 2014) air temperature and precipitation were recorded at 9.9˚C, 22.43 mm and 10˚C, 10.2 mm, respectively. The ten years mean maximum air temperature was recorded at 21˚C and precipitation was 1469.3 mm in July, and annual mean air temperature and precipitation were recorded at 16.5˚C and 441.2 mm, respectively (**Figure 2**).

The maximum and minimum soil temperatures of the study area were measured at 21.3˚C and 12.1˚C in October 2015, and 33.9˚C and 13.6˚C in April 2016, respectively. Maximum values of soil temperatures were measured at 15.9˚C, 21.3˚C and 20.2˚C in the morning, afternoon and evening, respectively, in October 2015. Similarly, the minimum values of soil temperatures were measured at 12.1˚C, 12.9˚C and 17˚C in the morning, afternoon and evening, respectively. The measurements in the evening on 29th October 2015 were not conducted due to unfavorable weather condition with cold wind and heavy rain. Likewise, in April 2016 the maximum and minimum values of soil temperatures measured were 17.40˚C, 24.67˚C, 33.93˚C, and 13.57˚C, 17.23˚C, 22.33˚C in the morning, afternoon and evening, respectively (**Figure 3**).

The maximum and minimum soil water content (SWC) was measured at 59.6% and 23.4% in October 2015, and 42.2% and 15.6% in April 2016, respectively. The average soil water content measured on 29th October was found highest (50.1%) because of the heavy rainfall in the previous night and day of measurements (**Figure 4**).

3.2. Soil Respiration and Soil Temperature

The soil respiration measured as a function of time calculated with the increase in concentration of CO_2 within the chamber (Equation (1)) showed an exponential relationship (Equation (2)) that was obtained between soil respiration and soil temperature at 5 cm soil depth (**Figure 3**). The soil temperature accounted 42.9% of soil respiration variability and the relationship was statistically significant ($p < 0.05$), in October 2015 (**Figure 3(a)**). And the temperature sensitivity of soil respiration (Q_{10}) value was obtained at 6.2. Whereas the soil temperature accounted 19.1% of soil respiration variability in April 2016 (**Figure 3(b)**), and the relationship was statistically significant ($p < 0.05$). The temperature sensitivity of soil respiration (Q_{10}) value was obtained at 1.4. The variation of soil respiration with the change in soil temperature by combining both measurements in October and April also showed significant ($p < 0.05$) exponential relationship, and the soil temperature accounted 23.3% of the soil respiration variability. In that case temperature sensitivity of soil respiration (Q_{10}) value (Equation (3)) obtained at 1.8 (**Figure 3(c)**).

3.3. Soil Respiration and Soil Water Content

The relationship between soil respiration and soil water content showed that soil respiration was increased with the increase in soil water content and peaked at some limit of 35% of the soil water content. In October 2015, the rate of soil respiration started to decrease after it reached the limit of soil saturation (35%) even when the soil water content was increasing (**Figure 4(a)**). The relationship was expressed as a negative significant exponential function ($R^2 = 0.50$, $p < 0.05$).

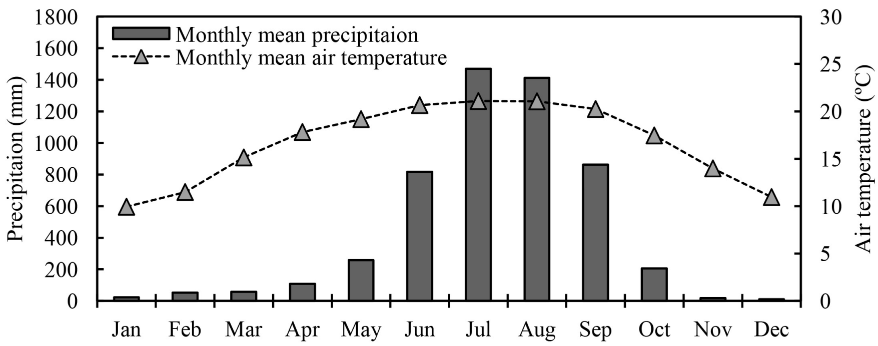

Figure 2. Monthly mean precipitation (bars) and air temperature (filled triangle) of the study area from 2005 to 2014 (Source: Department of hydrology and meteorology (DHM), Lumle).

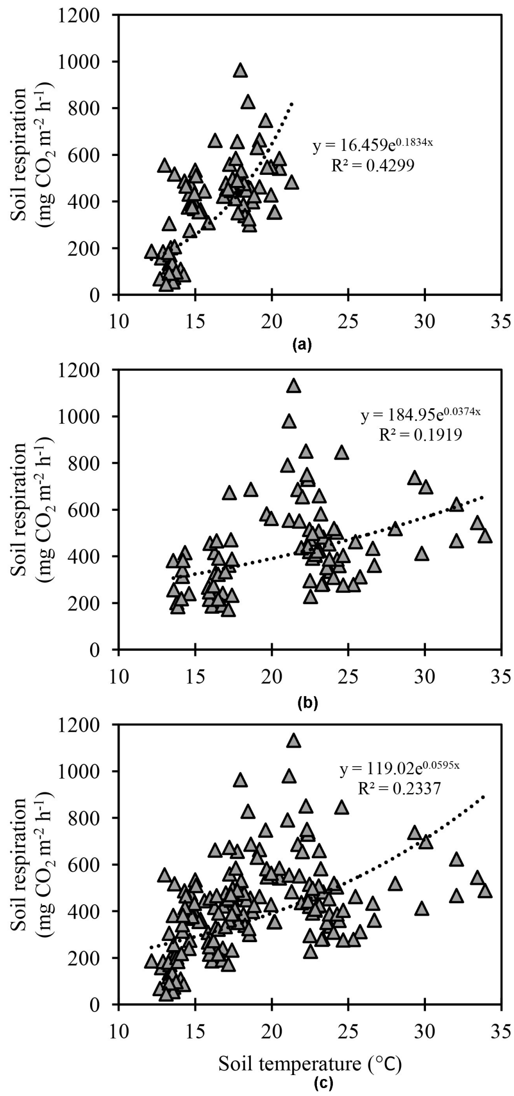

Figure 3. Relationship between soil respiration (mg CO_2 m^{-2} h^{-1}) and soil temperature (˚C) at 5 cm soil depth (a) October 2015, (b) April 2016 and (c) Combined (a) and (b).

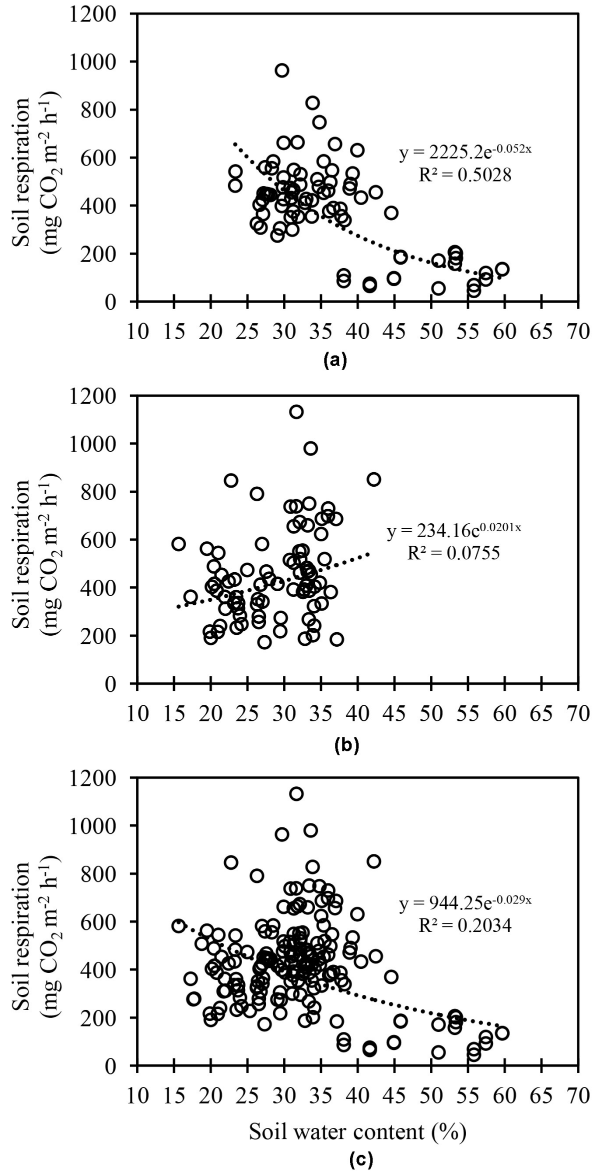

Figure 4. Relationship between soil respiration (mg CO_2 m^{-2} h^{-1}) and soil water content (SWC %) at 5 cm soil depth (a) October 2015, (b) April 2016 and (c) Combined (a) and (b).

In these cases, changes in soil water content ranged from 23.3% to 59.6%. A positive exponential relationship between soil respiration and soil water content was observed during the measurements in April 2016 and it was statistically significant (R^2 = 0.084, $p < 0.05$) and the changes in soil water content ranged from 15.6% to 42.2% (**Figure 4(b)**). During that period of measurements the soil water content did not cross above its higher limit (35%) so as to significantly suppress the soil respiration and the relationship was positive exponential upward trend. However, soil respiration and soil water content showed negative significant exponential function (R^2 = 0.20, $p < 0.05$) when both measurements in October and April were combined (**Figure 4(c)**). The maximum soil respiration was recorded between 30% and 35% of the soil water content measured throughout the study period.

3.4. Soil Respiration and Photosynthetic Photon Flux Density (PPFD)

Significant linear relationship was established ($R^2 = 0.376$, $p < 0.05$) between soil respiration and photosynthetic photon flux density (PPFD, Figure 5). Therefore, light (PPFD) accounted 37.6% of the soil respiration variability in this study. The maximum PPFD value was recorded at 1526.0 μ mol m^{-2} s^{-1} and minimum was recorded at 51.68 μ mol m^{-2} s^{-1} in October.

3.5. Soil Respiration of the Grassland

Maximum and minimum soil respiration values recorded at different time schedule in the morning, afternoon and evening in October 2015 showed that the values were comparatively lower in the morning gradually increased in the afternoon, and then slightly decreased in the evening. The evening values of soil respiration were higher than the morning in a clear day (Table 1). However, on 29th October the values decreased from the morning towards afternoon due to unfavorable weather condition with cold wind and heavy rainfall. Daily average soil respirations on 27th, 28th, and 29th October were 517.0, 430.5 and 123.4 mg CO_2 m^{-2} h^{-1}, respectively. Lowest average daily soil respiration was obtained on 29th October due to unexpected bad weather condition. The seasonal soil respiration in October was estimated (Equation (2)) at 356.97 mg CO_2 m^{-2} h^{-1} in October 2015. Similar trend of rising maximum and minimum soil respiration values from morning towards afternoon, and then decreased values in the evening were observed during measurements in April 2016 (Table 2), except on 20th April because the minimum value of the soil respiration was higher in the evening than the afternoon. Daily average soil respirations on 19th, 20th and 21st April 2016 were 525.3, 430.1 and 378.5 mg CO_2 m^{-2} h^{-1}, respectively. The seasonal soil respiration in April was estimated (Equation (2)) at 444.6 mg CO_2 m^{-2} h^{-1}.

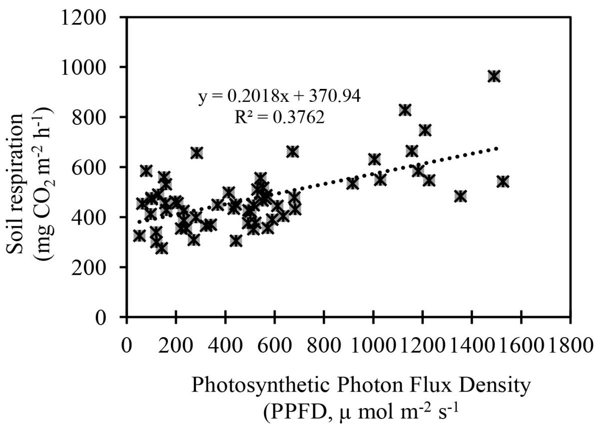

Figure 5. Relationship between soil respiration (mg CO_2 m^{-2} h^{-1}) and Photosynthetic Photon Flux Density (PPFD, μ mol m^{-2} s^{-1}) in October 2015.

Table 1. Maximum and minimum respiration with respect to the time, and daily average soil respiration (mg CO_2 m^{-2} h^{-1}) in October 2015 (n = 10).

Day October	Morning (7:00-9:00) am		Afternoon (12:00-2:00) pm		Evening (4:00-6:00) pm		Daily Average
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	
27th	555.71	305.22	963.42	483.4	656.5	339.44	517
28th	465.8	275.46	661.83	351.5	584.55	300.2	430.5
29th	201.66	65.82	184.27	45.3	NA	NA	123.4
Average							357.0

[NA = Not Available].

Table 2. Maximum and minimum respiration with respect to the time, and daily average soil respiration (mg CO_2 m^{-2} h^{-1}) in April 2016, n = 10.

Day April	Morning (7:00-9:00) am		Afternoon (12:00-2:00) pm		Evening (4:00-6:00) pm		Daily Average
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	
19th	415.87	184.31	1132.6	515.3	737.68	412.61	525.3
20th	466.27	187.06	850.98	283.13	846.31	312.21	430.1
21st	507.99	227.92	750.23	279.73	470.1	172.51	378.5
Average							444.6

The diurnal changes in soil respiration and the soil temperature of the grassland were very clear (**Figure 6**). Soil respirations were recorded higher during afternoon between 12:00 am and 2:00 pm than the morning and evening, and the values of soil respiration were always higher in the evening than the values observed in the morning (**Figure 6(a)**). The soil temperatures were recorded lower in the morning than the values observed in the afternoon, and there were not much difference in soil temperatures in the afternoon and evening (**Figure 6(a)**). The soil respiration measured on 20th April 2016 was higher in the evening than it was measured in the afternoon (**Figure 6(b)**), and the soil temperature as well was recorded high in that day of the measurement. This shows that the soil below the surface took some hours to cool down. The PPFD was recorded higher in the afternoon than the morning and evening at the time of higher soil respiration in clear day (**Figure 6(c)**). In evening PPFD was higher than the morning and lower than the afternoon measurements. Highest value of PPFD was obtained in the afternoon at 1230.8 μ mol m^{-2} s^{-1} on 27th and the lowest value was obtained in the evening at 118.0 μ mol m^{-2} s^{-1} on 28th October 2015.

This study showed clear daily variations of the soil respiration and soil temperature (**Figure 7(a)**). Daily soil respirations were comparatively higher at the time of higher soil temperatures, and the respiration was decreased on the day when soil temperature was remarkably low *i.e.* on 29th October 2016. However, differences in soil respirations were visible on those days when there were nearly equal or slightly different soil temperatures. Similar, daily variations of soil

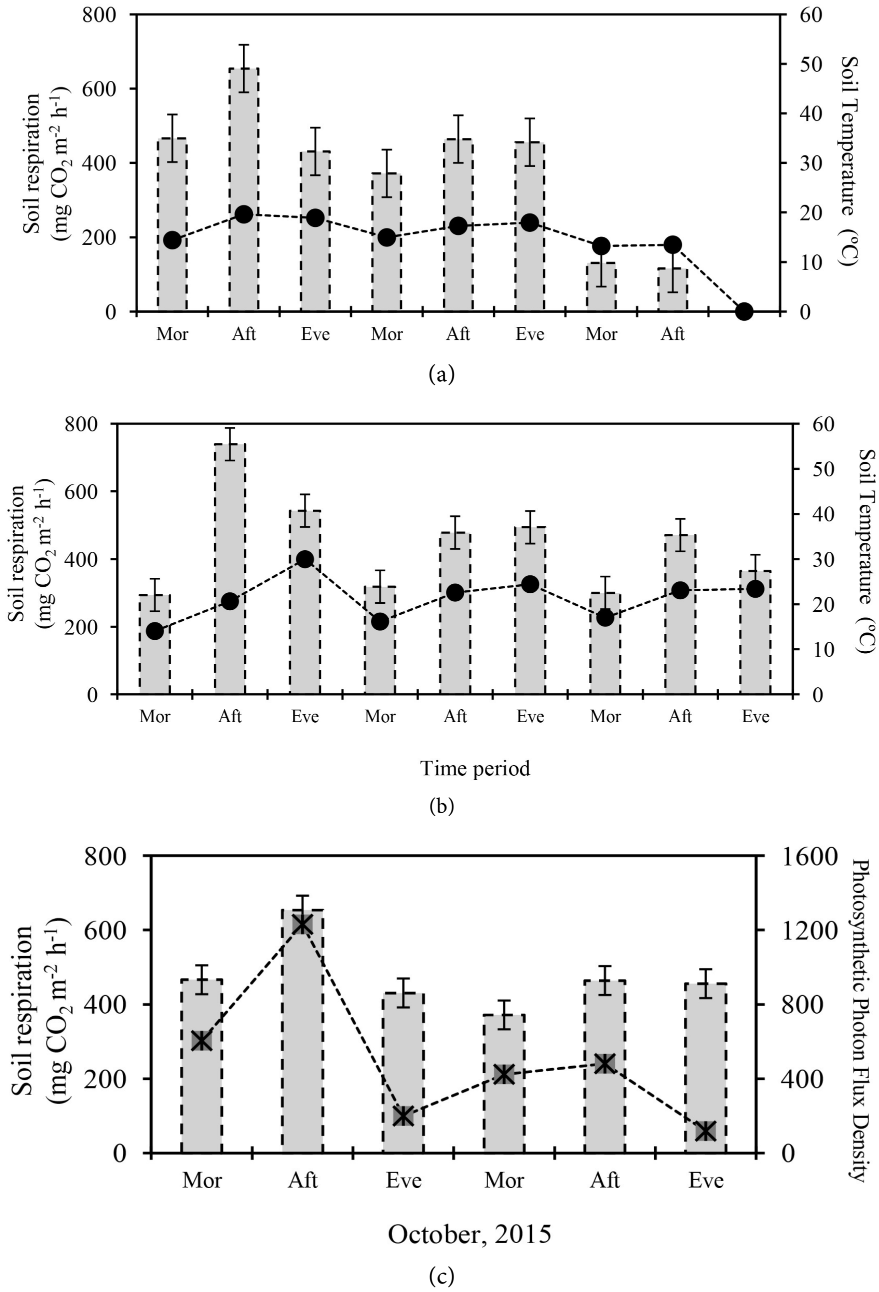

Figure 6. Diurnal changes in soil respiration (mg CO_2 m^{-2} h^{-1}) and soil temperature (˚C) at 5 cm soil depth (n = 10), (a) October, 27th, 28th and 29th in 2015, (b) April, 19th, 20th and 21st in 2016 (c) diurnal changes in soil respiration and Photosynthetic Photon Flux Density (PPFD, μ mol m^{-2} s^{-1}) on 27th and 28th October in 2015, n = 10. Bar, soil respiration; filled circle, soil temperature; filled square, PPFD; Mor, morning; Aft, afternoon; Eve, evening.

respiration and soil water content were observed in this study (Figure 7(b)). Slight difference in soil respiration and nearly equal soil water content values were obtained in the different days of the measurements. But, lowest soil respiration value was recorded on 29th October 2016 when soil water content was recorded highest among the date of measurements.

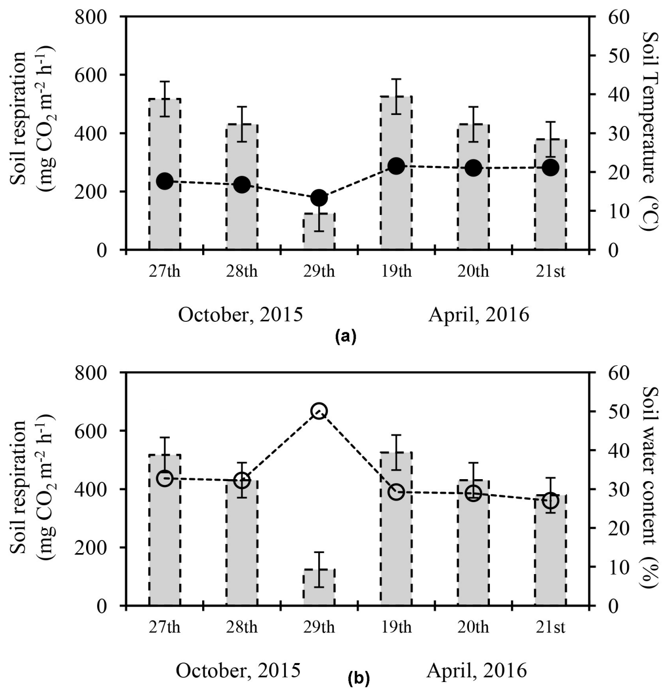

Figure 7. Daily Variations in (a) soil respiration (mg CO_2 m^{-2} h^{-1}) and soil temperature (˚C) at 5 cm soil depth and (b) soil respiration (mg CO_2 m^{-2} h^{-1}) and soil water content (%) in October, 2015 and April, 2016. Average of measurements is taken during morning, afternoon and evening. Bar, soil respiration; filled circle, soil temperature; open circle, soil water content.

3.6. Plant Biomass

Above-ground and below-ground plant biomasses were 231.1 g d w m^{-2} and 1538.8 g d w m^{-2} in October 2015, and 449.9 g d w m^{-2} and 349.0 g d w m^{-2} in April 2016, respectively (**Figure 8**). The dry weight of the above-ground plant biomass in October 2015 was half of the dry weight of above-ground plant biomass in April 2016. But, the dry weight of below-ground plant biomass in October 2015 was more than 4 times of its dry weight in April 2016. The bulk density of the grassland was calculated at 0.8 g cm^{-3}. The relationships between soil respiration and plant biomasses of the grassland were not observed in the study.

4. Discussion

4.1. Soil Respiration and Soil Temperature

The principal influencing factor affecting soil respiration was found to be soil temperature due to its common effect on soil micro climate and biological activity of below-ground organisms, thus it is indicated as major abiotic ecological driver of the ecosystem function. An exponential relationship (Equation (2)) was obtained between soil respiration and soil temperature (**Figure 3**). The eventual influence on soil respiration by the variation of soil temperature as observed in this study was similar to some researches [14] [20] [22] [60] [61] [62]. The soil

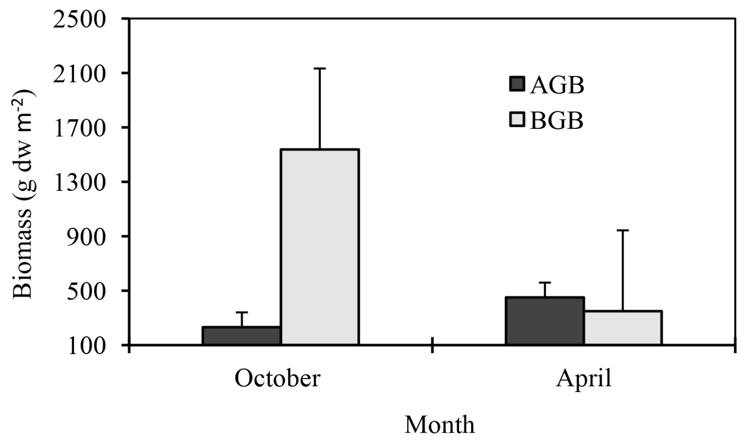

Figure 8. Above and below-ground biomass (n = 5) in October 2015 and April 2016.

temperature accounted 42.9% of soil respiration variability in October 2015 (**Figure 3(a)**) was much higher in comparison to the measurements (19.1%) in April 2016 (**Figure 3(b)**) was owing to the variation of the weather conditions *i.e.* very fine days with higher soil temperature to cloudy and rainy days with much lower soil temperature observed during the measurements period in October 2015. This showed that the temperature effect on soil respiration varies with the seasons. The physiological seasonal stability of the ecosystem during late growing season of the year in October showed due to observe the clear relationship between soil respiration and soil temperature than the ecologically active early growing season in April. The ecological activities of the grassland might be varying and readily fluctuating in early growing season in order to quote the growing period of the plants. Difference between maximum (21.3˚C) and minimum (12.1˚C) soil temperature in October was comparatively lower, and was half of the range than that was obtained (33.9˚C and 13.6˚C) in April 2016 but the variability of soil respiration in October 2015 was much higher than the variability of soil respiration in April 2016. This proved that soil respiration becomes more accountable and responsive to the change in soil temperature within lower range of soil temperature variation.

The lower (R^2 = 0.191) variability of soil respiration with the effect of soil temperature in April (**Figure 3(b)**) was owing to the dry soil condition and some rainfall events occurred few days before the measurements date after long dry days which might have caused an evident fluctuation in soil respiration of 1132.6 mg CO_2 m^{-2} h^{-1} at 21.4˚C and low soil respiration of 488.5 mg CO_2 m^{-2} h^{-1} at 33.9˚C. The periodic drying and wetting of soil has a pronounced influence in soil CO_2 evolution. When the soil is rewetted the activity of the microbes that were in a latent state in the dry soil increases and the release of air trapped in the soil pores contribute to an increase in soil respiration [63]. This phenomenon of increase in soil respiration by rewetting of dry soil was also observed by [64] [65] [66]. However, in contrast the rewetting of soil after reaching some limit suppresses the soil CO_2 evolution with the blockage of soil pores even at high temperature, and then starts emitting high CO_2 after the pores are reopened when the soil water evaporates. Apart from this, in April, some of the chambers also showed higher soil respiration throughout the measurements period that may cause the overlapping of the actual temperature effect on soil respiration, which was not clearly visible from the measurements. This could be solved with continuous digitalized measurements of soil respiration, soil temperature and precipitation for longer period of time to effectively determine the temperature and soil moisture effects on soil respiration. Our measurements provided an appropriate result to understand the temperature effect of soil respiration and seasonal water availability. Liebig *et al.* [67] observed that it could not established the correlation between soil respiration and soil temperature in summer due to the effect of stable soil temperature, and variations were found only in spring and autumn caused by rapid growth and senescence in semiarid grazing grassland, United States. This proved that this study was better enough to understand the overall soil respiration trend to the variations of its influencing factors in the temperate grassland.

The temperature sensitivity of the soil respiration (Q_{10}) values (Equation (3)), also defined as change in soil respiration at an interval of 10˚C soil temperature were estimated at 6.2, 1.4 and 1.8 in October, April and in combined measurements relatively varied with each other. However, the values were comparable to global carbon cycle research from 1.3 to 3.3 in the forests [68], 3.4 to 5.6 in temperate forests, USA [49], 2.23 in temperate grasslands [19], and 4.2 in beech forest [69]. The temperature sensitivity of soil respiration (Q_{10}) was lower in dry soil condition than in increased soil water availability and the effect was comparatively much pronounced in temperate region which was similar to previous observation [70], hence it might be the cause of variation in Q_{10} in our study. The soil water content was higher (59.9%) in October 2015 than in April 2016 (**Figure 4(a)**; **Figure 4(b)**). Besides, the soil respiration is more fluctuating at higher temperature range than the lower temperature range in grazing grassland [71]. The reason for relatively lower Q_{10} (1.4) and variation of soil respiration with the soil temperature (19.1%) was owing to the higher temperature range (13.6˚C - 33.9˚C) occurred in April 2016. Recent global data model had also revealed that temperate or cold region grassland vegetation had low effect of temperature on soil respiration rather than tropical region [67] [72]. High range (1 - 10) of Q_{10} values was reported in European forests; hence Q_{10} depends on ecosystem types, geographic location [73] and even on latitude for the same eco-types [75]. Thus, comparison of Q_{10} values is quite difficult, it is a very informative index and ecologically complicated for predicting soil carbon dynamics [76]. And it increases with soil drying and wetting due to below-ground activity of carbon mineralization and microbial cycle [20] [66] [76]. Not only soil temperature and soil moisture, Q_{10} values often are confounded with phenological processes and seasonal variations [77] as distinguished in this study and topo gradient [65] [78]. This proved many more associates govern the temperature sensitivity of soil respiration and truly determine its variability.

4.2. Soil Respiration and Soil Moisture

The effect of soil moisture on soil respiration was analyzed in this study for the account to evaluate its effect in ecosystem. Many studies have revealed that temperature and soil moisture are the major ecological abiotic factors, regulating determinants of soil respiration in terrestrial ecosystems from regional to the global scale [20] [43] [61] [62] [79]. The negative relationship (**Figure 4(a)**) between soil respiration and soil water content observed in October 2015 was liable to the rain events in first measurements day, after few days of clear weather. This type of negative relationship between soil respiration and soil water content with consequent restriction on soil respiration was observed in tropical rain forest of French Guiana [80]. Normally, the suppression of soil respiration due to rain events is caused by capillary effect where excess water in the soil limited gas production and transport. The soil respiration was generally increased with the increasing soil moisture up to some limit of soil moisture level. The upper limit of soil moisture to which the soil respiration increased was 35% and above that limit the soil respiration began to drop until the soil water content decreased and the negative curve was determined in October (**Figure 4(a)**). Similarly, in April 2016 lower soil moisture caused to suppress the soil respiration and that began to rise with increase in soil water content up to 35% that caused to result the positive exponential relationship between soil respiration and soil water content (**Figure 4(b)**). Reducing and enhancing of soil respiration under drought condition and high soil water level was observed in semiarid temperate grassland [81]. This showed that soil water availability has not only positive effect on soil respiration but also has negative effect with the decrease in rate of respiration, as observed in this study. Many studies have also obtained the results showing lower soil water availability reducing soil respiration [20] [31] [82]. However, combined effect of soil water content and soil temperature is more responsive to the variation of soil respiration [32] [61]. Soil respiration had minor sensitivity to temperature under low soil moisture level and it was more responsive to the temperature under high moisture level which was observed in this study (**Figure 4**), that was very much comparable to the previous studies [61] [82] [83]. However, the practical way of confounding soil respiration with soil moisture was derived using different equation indifferent ecosystems [79]. Therefore, when soil moisture is adequate to support biological activity; soil temperature becomes an important determinant for soil respiration as observed by Carbone *et al.* [25].

4.3. Soil Respiration and PPFD

A linear relationship between soil respiration and Photosynthetic Photon Flux Density (PPFD) was verified and more than 37% of the variability of soil respiration was explained by PPFD in the present study (**Figure 5**). The weather conditions on the days of measurements were not much similar which varied from sunny, cloudy to a rainy day. However, in our study the relationship was significantly clear to define the PPFD effect on soil respiration. Moreover, the variations of weather conditions during the measurements might be the cause of lower relationship between soil respiration and PPFD. The relationship observed in this study was nearly consistent with the research observed in an apple orchard in Northern Italy [84] *i.e.* in both scenarios less than 50% variability has been obtained. According to research conducted by Tang *et al.* [85] and Liu *et al.* [86], the effect of PPFD was likely due to the control of photosynthesis on carbon availability at the root system and soil respiration whose contribution to total soil respiration increases with light regimes, GPP, NPP and temperature. More detailed study might help to understand eco-physiological perspectives of soil respiration in temperate grassland.

4.4. Seasonal and Diurnal Change in Soil Respiration

The average soil respiration obtained during the measurements were 357.0 mg CO_2 m^{-2} h^{-1} and 444.6 mg CO_2 m^{-2} h^{-1} in October 2015 and April 2016, respectively, which represents the seasonal soil respiration in autumn and spring season of the grassland. The seasonal soil respiration value 444.6 mg CO_2 m^{-2} h^{-1} of this study is comparable to the value (417 mg CO_2 m^{-2} h^{-1}) obtained from the study conducted in Japanese Zoysia grassland in April [87] and also comparable to relatively high value (622 mg CO_2 m^{-2} h^{-1}) in May and (611 mg CO_2 m^{-2} h^{-1}) in October obtained from the study conducted in Zoysia grassland [14]. The variations seen in soil respiration during different seasons of this study might be comparable with the result of the study conducted in a perennial grassland for several years that showed lower soil respiration in the first four months (Jan.-Apr.) at lower soil temperature and soil respiration started increasing with increase in temperature until the temperature again started decreasing from autumn (October) towards winter [88]. The temperature response of soil respiration was obviously proven by the diurnal changes of soil respiration with lower values of soil respiration in the morning and evening at lower temperatures (**Figure 6(a)**; **Figure 6(b)**) and higher values of soil respiration in the afternoon with higher temperatures (**Table 1**; **Table 2**). The values obtained in this study were comparable to the soil respiration measured (49 - 358, 55 - 378 and 55 - 448 mg CO_2 m^{-2} h^{-1}) in tropical grassland, India [89] but lower maximum value (200 mg CO_2 m^{-2} h^{-1}) was reported in mixed grassland [90]. Therefore, the rate of respiration and its maximum and minimum values are mostly determined by temperature, soil moisture, light intensity, and mostly the seasonal factors, and they are the common and very sites specific for the variation of soil respiration.

4.5. Soil Respiration and Plant Biomass

Relationship between the soil respiration and plant biomasses were not noticeable in this study owing to limitations of continuously measured biomasses of the grassland throughout the year, and especially in the plants growing season. Grazing plays important role for carbon exchange and plant growth [60] [67] [91] [92]. Exclusion enhanced above-ground and below-ground biomass and litter, soil organic matter accumulation, and increased soil carbon storage and decreased bulk density [93] [94]. Different researches in the grassland ecosystem have revealed that below-ground plant productivity and soil moisture have direct common effects on soil respiration [95] [96]. According to Geng *et al.* [95], the variation in soil respiration with below-ground biomass was attributed at 80% among different sites, and below-ground biomass was directly correlated with root respiration of 31% - 51% in C3/C4 grassland [97], 14.5% - 62.62 % in rape field [96], 33% - 71% in perennial grassland [14] which is the major contributor of soil respiration in grassland. Limitations of research fund and the remoteness of the study area made it difficult to establish animal exclusion to measure the plant biomass and soil respiration with short measurement intervals throughout the year. The lower above-ground biomass in October 2015 (231.1 g d w m^{-2}) than in April 2016 (449.9 g d w m^{-2}) was attributed due to the seasonal variation, as October is the late growing season just after high grazing period following growing season and April is the early growing season with beginning of growing period with mild grazing effect. But, below-ground biomass was higher in October (1538.8 g d w m^{-2}) than April (349 g d w m^{-2}) (**Figure 8**). The below-ground plant biomass began to increase from early plant growing season (May) and reached its peak in autumn (October), then decreased throughout the winter as obtained in a perennial temperate Japanese grassland [9]. The plant biomass effect on soil respiration and in relation to the climatic factors could be the further research objective of this temperate grassland.

5. Conclusion

Soil temperature accounted 42.9%, 19.1% and 23.3% of soil respiration variability in October 2015 and April 2016 and both the measurements. The temperature sensitivity of soil respiration (Q_{10}) obtained was comparatively high in October (Q_{10} = 6.2) at lower range of soil temperature than the higher range in April (Q_{10} = 1.4) which showed that the temperature sensitivity of soil respiration decreased with increasing temperature range. Significant negative ($R^2 = 0.50$, $p < 0.05$) and positive ($R^2 = 0.084$, $p < 0.05$) exponential function were observed between soil respiration and soil water content in October 2015 and April 2016. Maximum soil respiration was observed between 30% and 35% of soil water content. Soil respiration showed significant linear relationship ($R^2 = 0.376$) with photosynthetic photon flux density (PPFD). Lower value of soil respiration measured during the morning and the higher value in the afternoon than in the evening were owing to the lower soil temperature in the morning and evening. Seasonal average soil respirations in October 2015 (357.0 mg CO_2 m^{-2} h^{-1}) were lower than April 2016 (444.6 mg CO_2 m^{-2} h^{-1}). The above-ground plant biomass in October 2015 (231.1 g d w m^{-2}) was half of its dry weight than April 2016 (449.9 g d w m^{-2}) and the dry weight of below-ground biomass in October (1538.8 g d w m^{-2}) was more than 4 times of its dry weight than in April (349.0 g d w m^{-2}). This study showed soil respiration variation in relation to the factors such as soil temperature, soil water content and photosynthetic photon flux density more likely in the prevailing climate change, signifying their importance in governing ecosystem function and carbon balance of the temperate grassland ecosystem.

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Conflicts of Interest

The authors declare that they have no competing interests regarding this research.

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