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ORIGINAL PAPER

DIURNAL AND SEASONAL SOIL CO_2 EFFLUX VARIATION IN SCOTS PINE (*PINUS SYLVESTRIS* L.) FORESTS IN THE EUROPEAN HEMI-BOREAL ZONE, LITHUANIA*

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Abstract

Various processes that occur in forest soils have significant roles in the forest carbon cycle and contribute to soil CO_2 efflux. Soil CO_2 efflux occurs via a combination between the metabolism of soil microorganisms and the respiration of plant roots. It releases around 10 times more CO_2 than all anthropogenic factors combined. For this reason, diurnal and seasonal carbon efflux rates were measured in Lithuanian Scots pine (*P. sylvestris*) forests to determine soil CO_2 efflux rates and the relative importance of various climatic factors that can influence below-ground conditions. The aim of this study was to determine the factors that have the strongest influence on variation in forest soil CO_2 efflux and the mutual influence between the factors, in order to attain a clearer understanding of C sinks. We took measurements of soil CO_2 efflux to examine diurnal and seasonal variation. For the continuous measurement of CO_2 efflux, an ADC BioScientific LCpro+ soil respiration analysis system was used. The main parameters that were measured were CO_2 efflux (μmol m^{-2} s^{-1}), air temperature (°C) in the chamber, soil temperature (°C) at a depth of 10 cm and water evaporation (mmol m^{-2} s^{-1}). The diurnal rate values fluctuated within a certain range (2.78-6.17 μmol m^{-2} s^{-1}), while seasonal variations fluctuated more, but the average CO_2 efflux rate was between 2 - 4 μmol m^{-2} s^{-1}. The highest CO_2 efflux rate recorded during daytime was from 16:00–20:00, and the lowest one was early in the morning, between 05:00–08:00. During the seasonal soil CO_2 efflux study, it was found that peak forest growth was related to peak efflux rates. These rates were observed from early to mid-June. The collected data suggest that SM (soil moisture) has a weaker connection with the efflux rate but had an impact on Ts (soil temperature), dampening its effect on soil CO_2 efflux. These results demonstrate that the soil CO_2 efflux of *P. sylvestris* forests in the cool climatic zone of Lithuania depend most heavily on soil temperature and soil moisture.

Keywords: carbon dioxide, soil temperature, soil CO_2 efflux, *Pinus Sylvestris*.

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INTRODUCTION

Carbon dioxide (CO_2) efflux from soil to the atmosphere is a major pathway in the global carbon (C) cycle and is one of the main sources of CO_2 in ecosystems between the biosphere and atmosphere (Buchmann 2000, Hartemink, Huting 2008, Wharton et al. 2012, Lingfei et al. 2020). Soil CO_2 effluxes are formed via a combination of the metabolism of soil microorganisms and the respiration of plant roots, and release around 10 times more CO_2 than all anthropogenic factors combined (Vanguelova et al. 2005, Fenn et al. 2010, Heinemeyer et al., Adair et al. 2011, Cueva et al. 2017, Roby et al. 2019). All of these processes together form soil CO_2 efflux, and can most often be found in organic surface layers of soil, where microorganisms decompose plant residues (Hibbard et al. 2005, Stoffel et al., Noe et al. 2010, Koster et al. 2011, Kopittke et al. 2012, Shabaga et al. 2015, Lee 2018, Silletta et al. 2019). There are also other factors that control these processes, such as the seasonal and daily variability of meteorological conditions (Silletta et al. 2019). The factors that affect soil CO_2 efflux and their relative importance cannot be determined without proper experimentation. In previous studies, different methods have been used to determine CO_2 efflux. Some of these methods were invasive and consisted of trenching or digging to exclude some components from the system (Fenn et al. 2010).

Forests are one of the most globally widespread ecosystems as they cover approximately 30% of the land surface of the Earth (Gustafsson et al. 2012, Qubaja et al. 2020). They contain the vast majority of global C pools – over 80% of all vegetation C is contained in forests, and around 40% of belowground soil C (Fang et al. 2014). Thus, the C dynamics of forest ecosystems are an important research topic. Forest ecosystems have a large capacity for C storage and vast productivity, meaning they have a significant role in the global C cycle and therefore in climate change (Vanguelova et al. 2005, Lingfei et al. 2020). Forest ecosystems play a crucial role in global climate change by reducing CO_2 emissions in the atmosphere from the burning of fossil fuels by approximately 30% (D'Andrea et al. 2020). In the near future, even small changes in the C balance of forest ecosystems and their connections with other ecosystems could have strong impacts on the global C cycle (Pan et al. 2011, Lindroth et al. 2020). To understand and determine the sources and sinks of C, more knowledge of the local variability of C is required (Kordowski, Kuttler 2010).

Soil CO_2 effluxes are caused by the activities of microorganisms and plant roots, and can be described as the impact of the living part of the soil. These activities may be altered by other factors that can increase or decrease CO_2 emissions from the soil. Such factors include photosynthetic properties of plants, soil properties, soil temperature (Ts), and soil moisture (SM) (Vanguelova et al. 2005, Lee 2018, Roby et al. 2019), the latter two factors being the most significant because they can influence other factors (Stoffel et al. 2010, Feizienė et al. 2012, Lindroth et al. 2018, Silletta et al. 2019). Soil temperature can regulate the metabolism of plant roots. The root systems of forest trees are composed of roots of different diameters and heterogeneous physiological traits and physical functions. In other forest ecosystems, root respiration plays a dominant role in the control of soil respiration. The results of various studies suggest that CO_2 efflux is positively related to litter and roots, and that root respiration accounts for a large proportion of total respiration. Previous studies indicated that root respiration contributes up to 65% of the total CO_2 efflux in boreal pine forest, in some cases reaching up to 90% (Hogberg et al. 2001). The amount of CO_2 efflux from the root respiration may vary depending on tree species or season (Epron et al. 1991, Lynch et al. 2013, Li et al. 2017, Wang et al. 2019). Most experiments that have been conducted thus far have shown that soil CO_2 efflux is connected with seasonal and daily patterns that were mainly controlled by soil temperature (Hartemink, Huting 2008). If we consider biological activities to be the only source of CO_2 efflux, then temperature is an important factor to be considered. Moreover, in certain climatic regions there are large temperature fluctuations between day and night, which affects biological activity and leads to variation in the CO_2 efflux rate. Furthermore, soil moisture can control CO_2 efflux in two different ways: by limiting aeration when soil moisture is high, thus limiting the diffusion of gases between soil and atmosphere, and by limiting the activities of microorganisms in decomposition processes when soil moisture is too low (Hibbard et al. 2005). There are many other factors that can moderate soil temperature and moisture, such as litter, fermentation, humus content, and other debris, which can prevent soil from experiencing extremes in temperature and moisture content (Khomik et al. 2010).

Approximately 33% of Lithuania is covered by forest, and around 34% of these forests are coniferous. The largest C stocks have been found to be stored in coniferous forest ecosystems and are considered to be highly stable despite changing conditions and various disturbances (Kopittke et al. 2012). In most coniferous forests, the largest area is occupied by pine stands. A handful of studies have been conducted to evaluate soil respiration and C dynamics in various pine stands, but these studies usually included only short measurement periods, such as on “clear days”, under the assumption that measurements made at a specific chosen time interval represent mean daily values (Feizienė et al. 2012, Cueva et al. 2017, Lindroth et al. 2020). Manual chamber measurements are most often taken to determine these values. Frequently, however, manual chamber measurements are impossible due to poor weather conditions or for other reasons, and therefore measurements are taken during daytime, generally during typical working hours, and thus diurnal variation is not recorded (Brændholt et al.).

As soil CO_2 effluxes are mainly determined by temperature, fluctuations in emissions can be observed during the day. Based on temperature fluctuations, the intensity of soil CO_2 efflux can be predicted (Shabaga et al. 2015, Zhiyong et al. 2015). As there are many unknown potential impacts of various environmental factors, there is little knowledge regarding the seasonal, daily, and annual trends in soil CO_2 effluxes (Lindroth et al. 2020). CO_2 efflux models that use soil temperature and soil moisture can help to determine CO_2 efflux intensity in different areas. However, these patterns are constrained by our understanding of the seasonal dynamics of soil CO_2 efflux, as CO_2 efflux has a very high temporal variability due to ever-changing environmental factors (Xu et al. 2011, Hu et al. 2016, Cueva et al. 2017, Throne et al. 2020).

In order to understand diurnal and seasonal fluctuations in the rate of CO_2 efflux, we established a study at experimental sites to record continuous measurements of the CO_2 efflux rate of the ecosystem. The aim of this study was to determine the factors that have the strongest influence on variation in forest soil CO_2 efflux and mutual influence between these factors, in order to attain a clearer understanding of C sinks. The objectives of this study were to investigate the diurnal and seasonal variations in CO_2 effluxes and their dependence on air, soil temperature and soil moisture in Scots pine (*Pinus sylvestris* var. *sylvestris* L., 1753) forests, and to determine other factors that may have impact the variations in soil CO_2 efflux rates such as vegetation cover, root respiration and forest type.

MATERIALS AND METHODS

Diurnal and sesonal studies was conducted at two different sites of mature Scots pine stands in central Lithuania. Lithuania lies in the southeastern coast of the Baltic Sea (Figure 1), and both sites were located near the city of Kaunas. Sites were approximately 100 m^2 in area. One of these sites was located 30 km west of the city, in the Meistine Forest (54°54′21″N, 23°33′17″E), and the other was situated 10 km northeast in the Pilenai Forest (54°57′31″N, 24°0′3″E). Both sites consisted of mature trees, with an average age of 100 years and an average height of 29 meters. The vegetation at the study sites was dominated by Scots pine (*Pinus sylvestris*). This region is situated in a cool temperate climate zone with moderate warm summers, moderate cold winters, and an average annual temperature of 8.8°C. The monthly mean temperatures in the coldest and warmest months are -3°C in January and 18°C in July, respectively. Most of the precipitation falls in summer time, and the mean annual precipitation is 675 mm. At ground level, vegetation is dominated by pedunculate oak (*Quercus robur* L.), common hazel (*Corylus avellana* L.), blueberry (*Vaccinium myrtilus* L.), lingonberry (*Vaccinium vitis-idaea* L.), red-stemmed feathermoss (*Pleurozium schreberi* (Brid.) Mitt.), and common nettle (*Urtica dioica* L.). The soil was classified as *Podzols* (Pitkaranta 2009) and was moderately developed. Podzols are typical soils of coniferous forest. This type of soil can occur

Fig. 1. Study site location

on almost any parent material but generally derives from either quartz-rich sands and sandstone or sedimentary debris from magmatic rocks, provided there is high precipitation. Most Podzols are poor soils for agriculture due to the sandy portion, resulting in a low level of moisture and nutrients. The soil consisted of two main layers, the first being a litter layer that varied from 5 to 8 cm in depth. The litter layer consisted of three sub-layers: in the top layer there were sparsely decomposed needles, leaves, small saplings; in the second layer the particles were moderately decomposed; and in the third layer all particles were decomposed. The second layer was a mineral sandy horizon that continued to 1 m in depth or more and consisted of four different sub-layers mostly of sand.

Measuring soil CO_2 efflux and H_2O net exchange rate

For the continuous measurement of CO_2 efflux, an ADC BioScientific LCpro+ soil respiration analysis system was used (made by ADC BioScientific Limited, the UK). It uses a steady-state through-flow and an open dynamic chamber. The system used the following formula to calculate CO_2 efflux (± 2% accuracy):

$$NCER = u_s\ (-\Delta c) \qquad (1)$$

where: NCER – CO_2 efflux (μmol s^{-1} m^{-2}), u_s – the molar flow of air per square meter of soil and Δc is the difference in CO_2 concentration through a soil pot (dilution corrected to μmol mol^{-1}).

Depending on the area of study, measurements were taken from the forest soil surface at multiple locations (generally 3). The LCpro+ with its soil pot is specifically designed for portability and field use. This system also measures soil temperature and chamber air temperature (±0.2 accuracy), photosynthetically active radiation (PAR) (±2% accuracy), and atmospheric pressure (±2% accuracy).

When taking measurments for the diurnal component of the study, once the measurement location was selected, the programming console was connected to the soil pot chamber. A metal ring with a diameter of 10 cm was inserted into the selected location, and the soil pot chamber was then attached to it. The ring was inserted perpendicularly to the soil at a depth of approximately 10 cm and then left to collect data. During the measurements, CO_2 was supplied from the atmosphere, from an altitude of 2-2.5 m, by a telescopic probe. A sample was taken every minute in the soil pot chamber, and after ten minutes of measurement averages were calculated to avoid any inclusion of the device's deviations and inaccurate measurements. Continuous measurements were performed for several days, depending on weather conditions, and in total there were four sets of samples taken. One set was taken from 24-26 July 2018 (study period 1), another from 24-25 August 2019 (study period 2), and two sets were taken in 2020, one from 21-22 July (study period 3) and one from 11-13 August (study period 4). The sampling period varied from 24 to 48 hours. The main parameters that were measured were CO_2 efflux (μmol m^{-2} s^{-1}), air temperature (Ta, °C) in the chamber, soil temperature (Ts, °C) at a depth of 10 cm, and water evaporation (mmol m^{-2} s^{-1}).

For the seasonal component of the study, PVC rings were used instead of metal rings. Ten plastic rings were installed in the soil at least 3 m away from each other at permanent sites two weeks before measurements were taken, creating study sites approximately 100 m^2 in area. At the study sites, every separate measuring pot monitored a period of 15 minutes so that all of the parameters could settle and could then be recorded. After this, readings were recorded manually and when the necessary data had been recorded then all of the equipment was moved to another stationary pot location. Measurements were taken 6 to 8 times during each season. Every time at least 10 measurements were taken, creating 60 to 80 samples for every study period. The study time of 11:00-17:00 was chosen because the environmental parameters change the least during this period. The same parameters were measured each time, and measurements were taken in three years (2013, 2019, and 2020). Studies were conducted during the plant growing period from May 11 to August 20 (in 2013, some extra measurements were taken in October and November).

The H_2O exchange rate was measured with the same ADC BioScientific LCpro+ system as CO_2 efflux. The H_2O measurement was taken with two high quality humidity sensors and then calculated from the following formula:

$$W_{flux} = \frac{\Delta e u_s}{p} \tag{2}$$

where: W_{flux} – the net soil H_2O exchange, u_s – the molar flow of air per square meter of soil, mol m^{-2} s^{-1}, Δe – the differential water vapor concentration in mbar, dilution corrected, and p – the atmospheric pressure in mBar.

To understand better the relationships between soil CO_2 efflux and its drivers (Ta, Ts), we created a simple statistical model of Q_{10}, which is a temperature coefficient that represents the factor by which the rate (R) of a reaction increases for every 10-degree rise in temp. (T). The rate (R) may represent any measure of the progress of a process. This factor was calculated from

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)} \tag{3}$$

where: Q_{10} – the factor by which the reaction rate increases when the temperature raises by ten degrees, R_1 – the measured reaction rate at temp. T_1, R_2 – the measured reaction rate at temp. T_2, and T_1 has to have a lower value than T_2.

Measuring soil temperature and moisture content

The temperature (Ta) in the soil pot chamber and the soil temperature (Ts) at a depth of 10 cm were obtained with the LCpro+ for the diurnal study of soil CO_2 efflux as well. During the seasonal study, soil temperature (Ts, °C) and soil moisture (SM, %) data at a depth of 10 cm were obtained using the HH2 moisture meter. The WET (water content, electrical conductivity and temperature) device directly measures soil permittivity, bulk electrical conductivity and temperature. From these, and with the use of specific soil calibration tables and equations, the HH2 calculates volumetric SM.

When the measurements were taken with the LCpro+, a WET sensor was inserted into the soil in the same location and the Ts and SM measurements were recorded. The sensor was inserted into the litter horizon to a depth from 5 to 8 cm. The same measurements were then repeated in other soil pot spots.

Statistical analysis

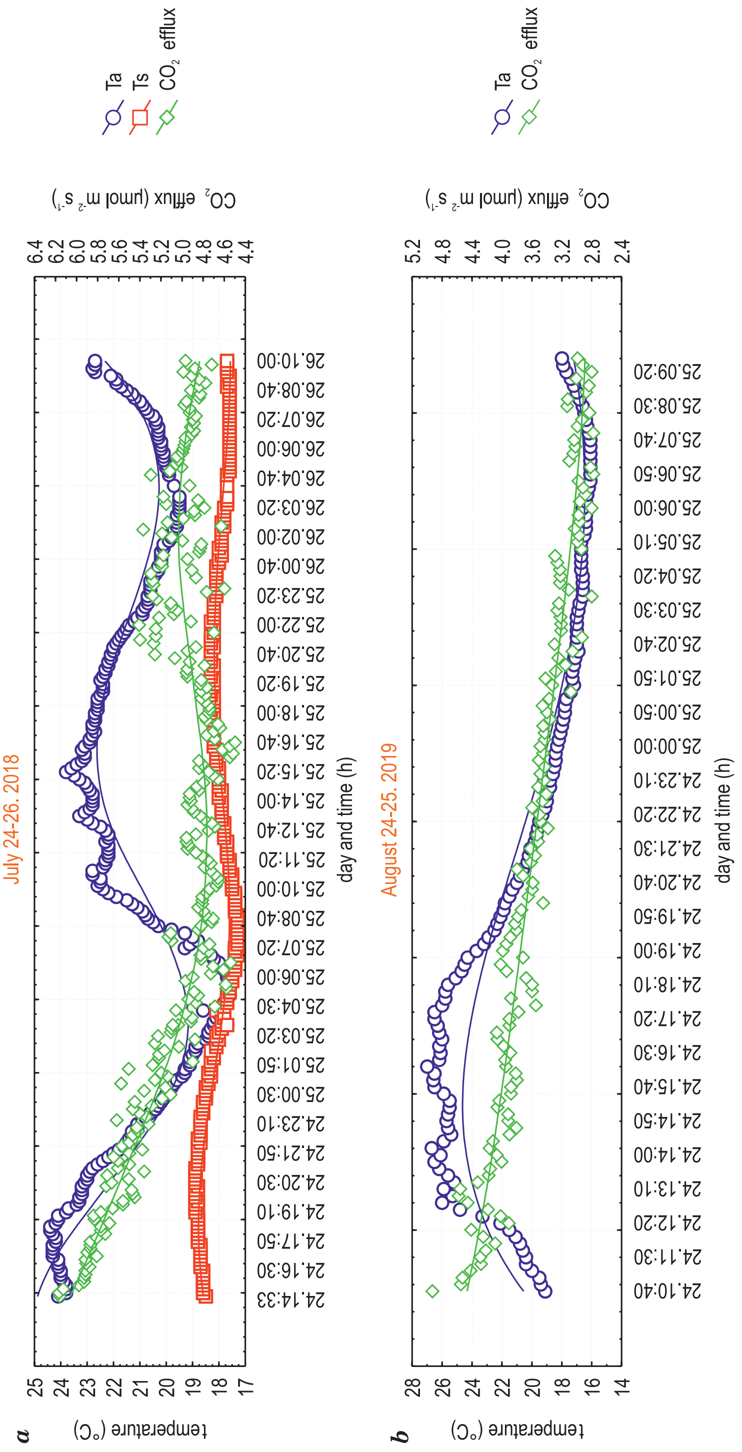
The measurements provided by the automated soil pot chamber were averaged for every ten minutes to obtain accurate diurnal data. These data were cleared and processed using Microsoft Excel. Diurnal variation was tested using multiple regression analysis. Seasonal differences were tested using the mean daily soil CO_2 efflux for each plot on a given sample date, using a two-way analysis of variance. Correlations and relationships between data were tested using ANOVA. Data were used to interpret the simple impacts associated with significant interactions between the effects of date, day, and night. Significance tests were conducted using the Fisher's unrestricted least significant difference (LSD) test. Significant differences were assessed at the level of $p<0.05$ and analyses were performed using the Statistica 8 statistical software.

RESULTS

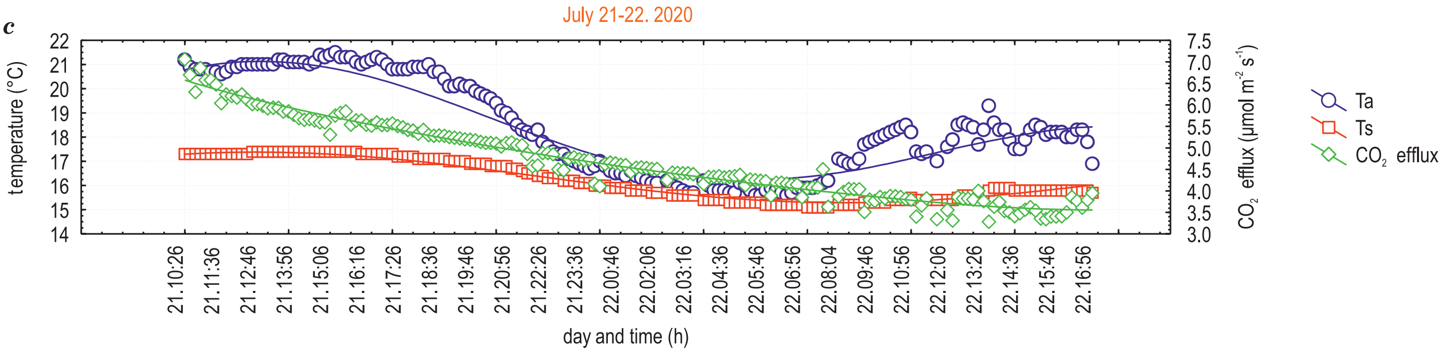
Diurnal soil and air temperature variations

The diurnal variation of air (Ta) and soil (Ts) temperature at pine forest stands in all of the years studied (July 24-26, 2018 total replicates 2570; August 24-25, 2019 total replicates 1363; July 21-22, 2020 total replicates 1714; August 11-13, 2020 total replicates 2699) is shown in Figure 2. In 2018, the minimum diurnal Ta was observed from 05:00 to 06:00. In some cases, the minimum values were obtained from 03:00 to 04:00. Maximum diurnal Ta values were observed from 15:00 to 18:00. The lowest Ta recorded was 17.6°C, highest 24.4°C. The minimum diurnal Ts was observed from 05:00 to 09:00. Meanwhile, the highest recorded Ts was recorded from 18:00 to 20:00 (Figure 2*a*). The lowest Ts observed was 17.3°C and the highest was 18.9°C. During 2019 (Figure 2*b*), the lowest Ta recorded was between 07:00 and 08:00 and the lowest was observed from 16:00 to 17:00. In July 2020, the second day of the experiment was interrupted by rain (Figure 2*c*). The lowest Ta was observed from 05:00 to 06:00 and the highest was observed from 15:00 to 16:00, with temp. of 15.6°C and 21.5°C, respectively. The lowest recorded Ts was from 07:00 to 08:00. The highest observed Ts was from 13:00 to 16:00 and held constant despite rain. In August 2020 (Figure 2*d*), in all study periods the minimum Ta recorded was around 06:00 and the maximum Ta was observed around 16:00. The lowest and highest Ta were 12°C and 24.3°C, respectively. The minimum Ts in all study periods was observed between 07:00 and 09:00, while the maximum Ts was observed between 18:00 and 20:00. The lowest Ts was 13.6°C and the highest Ts was 18.4°C.

The diurnal patterns of Ta and Ts were similar throughout the study period. Over the course of the measurements, the Ts changed less and stayed more constant than Ta, but each of these factors still displayed a correlation whit CO_2 efflux (2018, r=0.48; July 2020, r=0.91; August 2020, r=0.87; $p<0.05$). Overall, the data suggest that Ts has a greater impact on soil processes from the evening until the early morning.


a
July 24-26. 2018
Ta
Ts
CO_2 efflux
temperature (°C)
CO_2 efflux (µmol m^{-2} s^{-1})
day and time (h)
b
August 24-25. 2019
Ta
CO_2 efflux
temperature (°C)
CO_2 efflux (µmol m^{-2} s^{-1})
day and time (h)

c



d

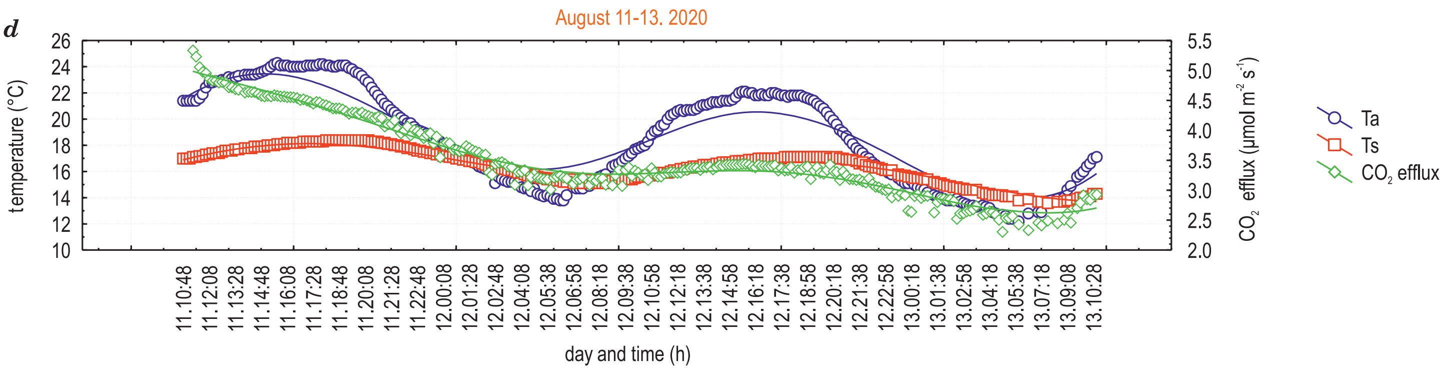


Fig. 2. Relationship of CO_2 efflux with soil temperature at a depth of 10 cm and temperature in a soil pot chamber: *a* – results from July 24-26, 2018, *b* – results from August 24-25, 2019, *c* – results from July 21-22, 2020, *d* – results from August 11-13, 2020. Ta – air temperature, Ts – soil temperature. Soil temperature data are missing from the 2019 study because of a missing temperature probe

Diurnal CO_2 efflux variations

During this study, the hourly soil CO_2 efflux varied significantly ($p<0.001$) – Figure 2. Soil CO_2 efflux rates were significantly higher during the daytime than at nighttime, and the peak soil CO_2 efflux in all trials was observed between 16:00-20:00 hours. In 2018, the mean CO_2 efflux rate was 6.17 μmol m^{-2} s^{-1} at the beginning of measurements at 15:30 CO_2 efflux was high because of disruption of the soil (Figure 2*a*). After some time around 20:00, efflux settled down at 5.2 μmol m^{-2} s^{-1} and varied in similar parameters up until 22:00 after which the CO_2 efflux gradually decreased. The lowest rate of 4.71 μmol m^{-2} s^{-1} was recorded at 06:30, although there was a sudden change in the weather at 10:10 accompanied by an observed soil CO_2 efflux rate of 4.68 μmol m^{-2} s^{-1}. On the second day, due to the changes in the weather, there were no significant changes in the soil CO_2 efflux rate. During the daytime, the highest average rate was 4.85 μmol m^{-2} s^{-1}, recorded at 11:40-12:30. In the evening, the efflux rate started to rise until it reached 5.41 μmol m^{-2} s^{-1} at 22:00, after which it remained similar until the end of the next morning. The combination of air and soil temperatures led to significant variations in CO_2 efflux.

In 2019, the 24-hour data recorded showed a gradually decreasing soil CO_2 efflux rate (Figure 2*b*). The highest rate observed was 4.58 μmol m^{-2} s^{-1} at 13:00, which decreased until 06:50, when the minimum value of 2.78 μmol m^{-2} s^{-1} was observed. After that, the rate stabilized, giving an average of 2.99 μmol m^{-2} s^{-1}.

In July 2020 (Figure 2*c*), the average soil CO_2 efflux rate of was 4.65 μmol m^{-2} s^{-1} and the highest value was 7.06 μmol m^{-2} s^{-1}, recorded at 11:00. Due to the rain on the second day, there were no significant variations in the rate of soil CO_2 efflux. The minimum rate value of 3.63 μmol m^{-2} s^{-1} was recorded at 08:00. After that, the efflux rate did not increase, but fluctuated between values of 3.00 and 4.05 μmol m^{-2} s^{-1}, with an average rate of 3.65 μmol m^{-2} s^{-1}, from the morning until the end of the experiment.

In August 2020 (Figure 2*d*), the average efflux rate of the entire study was 3.47 μmol m^{-2} s^{-1}. Soil CO_2 efflux rates showed significant hourly changes and were closely related to temperature changes. On the first day, a high rate of 4.81 μmol m^{-2} s^{-1} was recorded at 12:48. After some time around 16:00, the efflux settled down at 4.5 μmol m^{-2} s^{-1} and varied between 4 and 4.5 μmol m^{-2} s^{-1} until 20:00.The lowest value of 3.01 μmol m^{-2} s^{-1} was recorded at 05:48. In the morning, the soil CO_2 efflux rate started to rise and on the second day reached its maximum of 3.44 μmol m^{-2} s^{-1} at 15:38, after which it varied from 3.2 to 3.44 until 20:00. The lowest recorded value on the second day was 2.31 μmol m^{-2} s^{-1} at 04:48.

The relationship between diurnal soil and air temperature and CO_2 variations

The correlation of Ta with soil CO_2 efflux was high in most cases but remained lower than that of Ts. In the 2018 study period, rapid fluctuations in Ta were observed, while CO_2 efflux was more constant. Thus, Ta and CO_2 efflux displayed a low level of correlation (Table 1). Ts was more constant over all study periods and its correlation with efflux was stronger. In 2019, Ta had a high positive correlation with the soil CO_2 efflux. The data from 2020 show a strong correlation between Ta, Ts and soil CO_2 efflux, with the correlation between Ta and CO_2 efflux weaker than that of Ts and CO_2 efflux. Thus, Ts could explain more variation in the soil CO_2 efflux of the forest stands. The determination coefficient for Ta was r^2=0.11, r^2=0.57, r^2=0.52 and r^2=0.62 for study periods 1 to 4, respectively. Ts determination coefficient was r^2=0.54, r^2=no data, r^2=0.77 and r^2=0.66 for study periods 1 to 4, respectively.

Table 1

Correlations between CO_2 efflux and Ta, Ts, and H_2O for the four sampling periods on the sampling dates

Year	Ta	Ts	H_2O
2018	0.35	0.73	0.61
2019	0.75	–	0.67
July 2020	0.72	0.88	0.63
August 2020	0.78	0.81	0.77

Ta – air temperature, Ts – soil temperature, correlations are significant at p<0.05

Diurnal variations in soil H_2O exchange rate and its relationship with soil CO_2 efflux

The mean average net soil H_2O exchange rate in all studies across all years showed diurnal fluctuations (Figure 3) that mimicked the patterns of temperatures (Ta, Ts). In 2018, mean evaporation was 0.10 mmol m^{-2} s^{-1} (Figure 3*a*). The maximum level of evaporation recorded was 0.18 mmol m^{-2} s^{-1}, observed at 17:30-18:10. The minimum evaporation in 2018 was observed from 08:00 to 09:00. Readings were 0.06 mmol m^{-2} s^{-1}. In 2019 (Figure 3*b*), the highest H_2O exchange rate was recorded in the daytime, with a value of 0.27 mmol m^{-2} s^{-1} at 16:10. The lowest rate was in the morning, at 0.05 mmol m^{-2} s^{-1} from 09:20-09:40. Similar patterns were recorded in July 2020: The maximum rate observed was 0.25 mmol m^{-2} s^{-1}, recorded several times in the evening from 17:30 to 20:00. The minimum value was 0.1 mmol m^{-2} s^{-1}, observed at 07:00 (Figure 3*c*). In August 2020, there were significant variations in net soil H_2O exchange rate (Figure 3*d*). The maximum observed H_2O exchange rate was 0.24 mmol m^{-2} s^{-1} from 19:00 to 20:00. The minimum observed variation was fluctuating between 0.07-0.08 mmol m^{-2} s^{-1} from 07:00 to 08:00. During all of the study periods, the weather had a significant im-

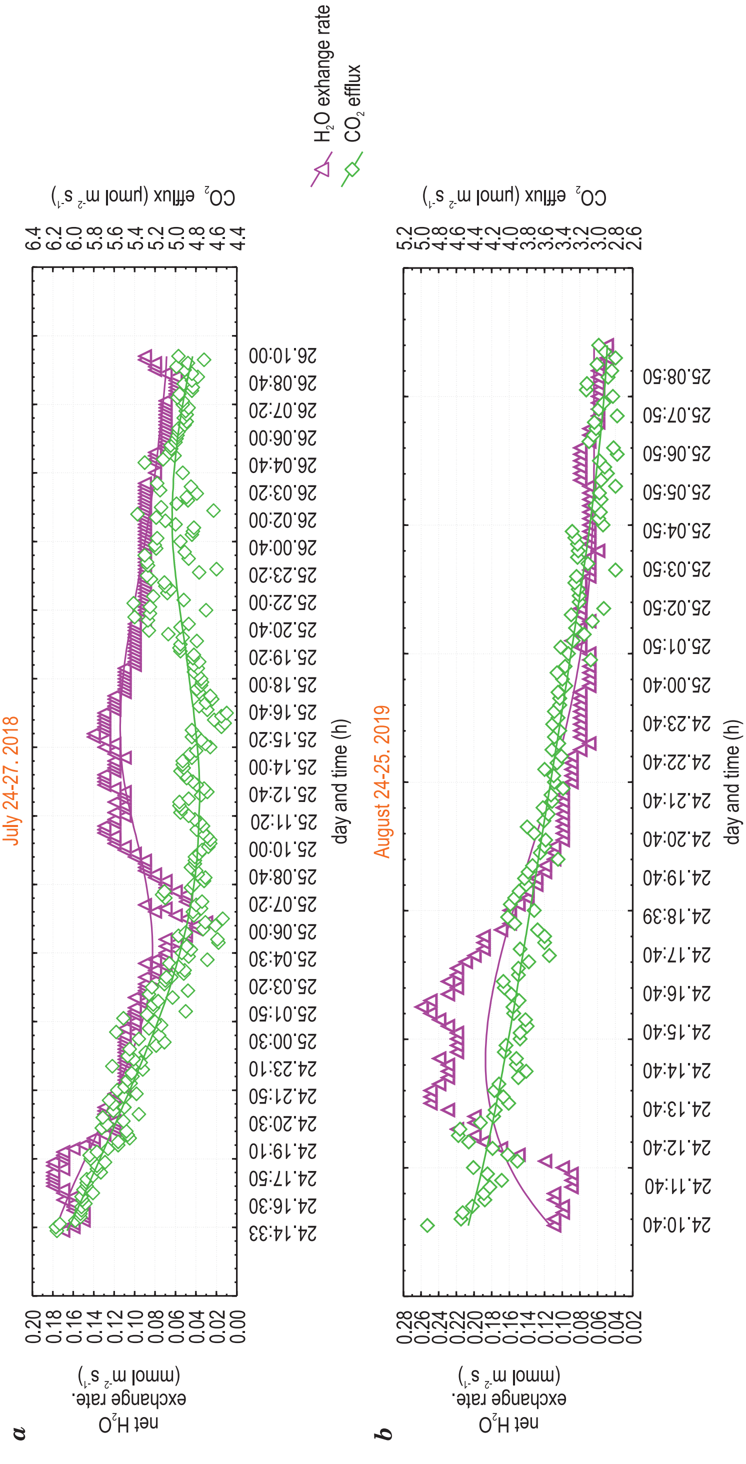

a
July 24-27. 2018
net H_2O exchange rate. (mmol m^{-2} s^{-1})
CO_2 efflux (µmol m^{-2} s^{-1})
day and time (h)
H_2O exhange rate
CO_2 efflux
b
August 24-25. 2019
net H_2O exchange rate. (mmol m^{-2} s^{-1})
CO_2 efflux (µmol m^{-2} s^{-1})
day and time (h)

Fig. 3. Relationship of CO_2 efflux and H_2O exchange rate: *a* – study from July 24-27, 2018, *b* – study from August 24-25, 2019: *c* – study from July 21-22, 2020, *d* – study from August 11-13, 2020

pact on water evaporation rates. During the monitoring, it was observed that the maximum evaporation occurs 3 to 4 h after the maximum soil CO_2 efflux rate. The H_2O exchange rate increased during daylight hours and peaked around the same time as air and soil temperatures. Afterwards, these rates decreased overnight, with the lowest value usually observed in the early morning from 07:00-09:00. Nevertheless, the soil H_2O exchange rate had a stronger correlation with Ta (average mean correlation of all experiments r=0.88, correlations significant at p<0.05) than with Ts (r=0.82), albeit not by a wide margin. The correlation between water evaporation and soil CO_2 efflux was lower (r=0.67, p<0.05).

Seasonal variation in temperature and soil moisture

Ta and Ts values varied throughout the different years of study (Figure 4). The lowest values of Ta and Ts recorded in 2013 were 8.4°C and 6.8°C, respectively, on November 12 (Figure 4*a*). In 2019, the lowest recorded Ta was 13.7°C and the lowest recorded Ts was 11.4°C, both of which were recorded on May 11 (Figure 4*c*). In 2020, the lowest temperatures were recorded in July due to the unusually cold weather, when the lowest Ta was 19°C and the lowest Ts was 17°C (Figure 4*e*).

The Ta and Ts at a depth of 10 cm peaked around the same time each year. In 2013, the highest Ta was 27.6°C and the highest Ts was 23.5°C, both being recorded on May 17. In the beginning of summer 2019 there was a huge spike in temperature, with the highest Ta of 29.9°C and Ts of 27.2°C being recorded on June 8, unusually warm for the time of year. In 2020, the highest temperatures were recorded on June 19. The day was very hot and the highest Ta and Ts was 33.1°C and 28°C.

Throughout the years studied, SM fluctuated because of the time of year and the weather. There was no consistent pattern of SM observed. In the data recorded in 2013, the study average was 12.7% (Figure 4*a*). The lowest SM rate was recorded in late spring on May 17, with a reading of 8.8%. After that, SM climbed during the summer until, on July 19, it reached its peak of 19.3%, after which it began to drop until it peaked again in late fall. On November 12, the SM was 25.9% because of heavy rainfall and the absence of enough heat to evaporate the resultant moisture. At this study, SM had negative correlation with both Ta and Ts r=0.34 and r=-0.35 respectively (p<0.05).

In 2019, the spring SM had a high average: on May 11, average daily SM was 15.7% (Figure 4*c*). This figure remained similar until the end of the month and then dropped significantly in June. Throughout the measuring period, SM rose and fell, but by the end of the summer SM began to rise. On August 18, the average SM was 20.9%, the highest average over all periods measured. During this study, SM and temperature had very weak correlation. Ta r=-0.22 and was significant at p<0.05, Ts r=-0.14 correlation was not significant.

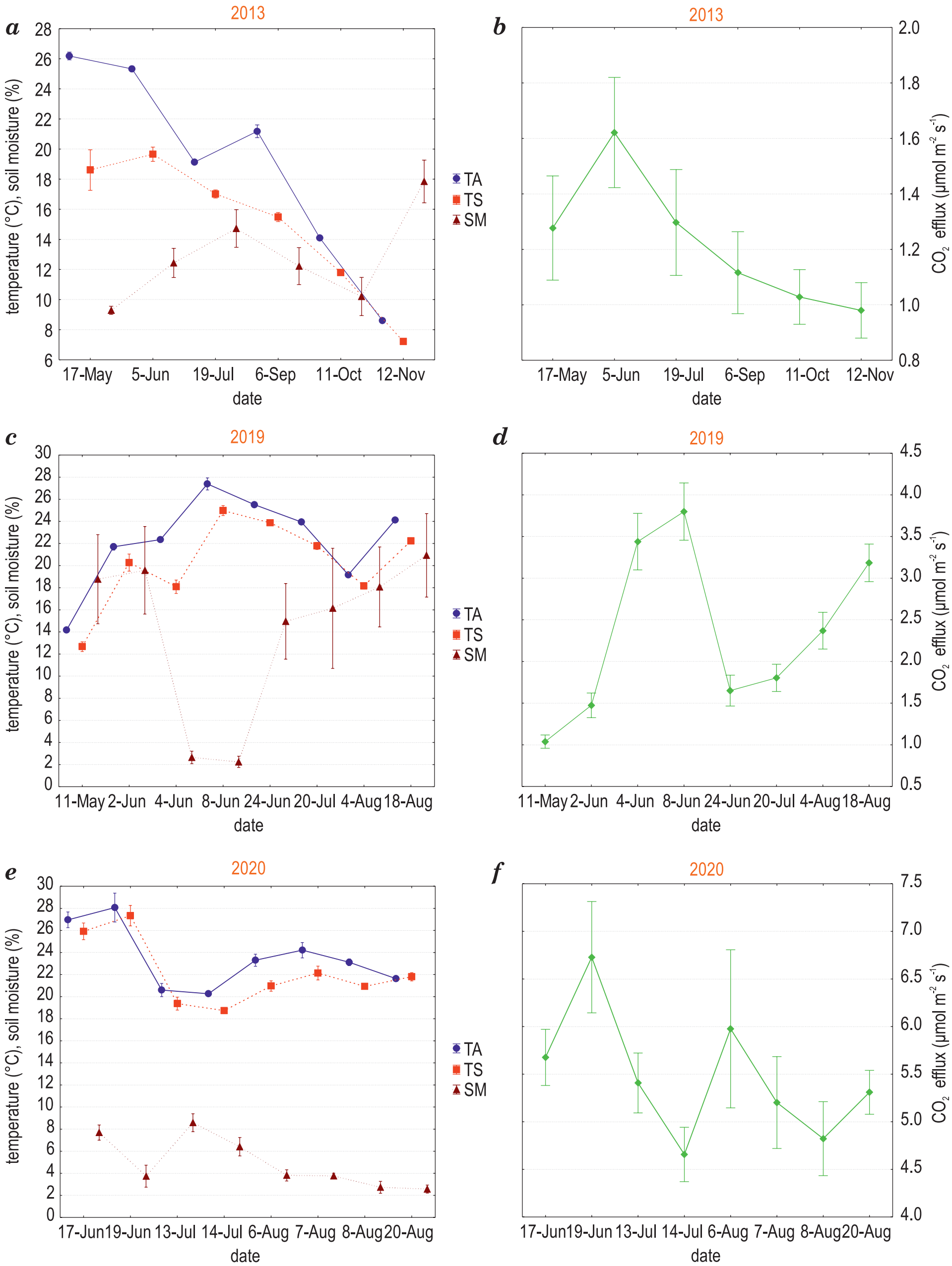


Fig. 4. Seasonal variations of soil pot chamber temperature (Ta), soil temperature (Ts), soil moisture (SM) and soil CO_2 efflux in different years: (*a*) and (*b*) are from 2013, (*c*) and (*d*) are from 2019, and (*e*) and (*f*) are from 2020

2020 was a very dry year, with an average SM reaching just 4.8%. The highest daily average was recorded on July 13 (Figure 4*e*), albeit still low at 8.5%. From that point onwards, SM decreased. In August, measurements were made on four different days in a row, where the average SM was only 3.2%. Throughout all of these experiments, no significant correlation was recorded between temperature and SM.

Variations in seasonal soil CO_2 efflux rate and the relationship between temperature and soil moisture

In pine stands, soil CO_2 effluxes in all of the years studied peaked at almost the same time, in early or middle June (Figure 4). For all forest stands, soil CO_2 efflux showed significant seasonal variation, and variations were similar to changes in temperature (Figure 4). The highest average efflux recorded was on June 5, with a value of 1.62 µmol $m^{-2}\,s^{-1}$ in the 2013 season. The lowest was recorded in late autumn, with 0.32 µmol $m^{-2}\,s^{-1}$ on 12 of November 2013. After reaching its highest rate, soil CO_2 efflux decreased until the last sampling date of the 2013 season (Figure 4*b*). During the 2019 season, the highest efflux rate of 3.8 µmol $m^{-2}\,s^{-1}$ was recorded on June 8 and the lowest efflux rate of 0.66 µmol $m^{-2}\,s^{-1}$ was recorded on June 24. In the 2019 season, after the highest efflux rates were recorded, they decreased and then started to increase until the last sampling date (Figure 4*d*). In the 2020 season, the highest soil CO_2 efflux rate of all experiments was recorded, with a value of 6.72 µmol $m^{-2}\,s^{-1}$ recorded on June 19. During the 2020 season, after seasonal highs the soil CO_2 efflux rate decreased and then increased multiple times during the sampling period (Figure 4*f*). The lowest value of 3.46 µmol $m^{-2}\,s^{-1}$ was recorded on July 14.

The Q_{10} showed similar values over each study period. The lowest value of Q_{10} CO_2 efflux versus Ta during all experiments was 1.1 and the highest value was 2.5. The CO_2 efflux versus Ts showed slightly higher values, with the lowest Q_{10} being 1.3 and the highest was 3.5.

The seasonal soil CO_2 efflux rate had a strong correlation with temperature in all of the forest stands throughout all of the periods under study but had a low level of correlation with SM (Table 2). However, when the SM

Table 2

Soil CO_2 efflux correlations with mean Ta, Ts, SM, and H_2O exchange rate for the four sampling periods on the sampling dates

Year	Ta	Ts	H_2O	SM
2013	0.33	0.33	0.52	0.04*
2019	0.52	0.40	0.62	−0.29
2020	0.32	0.43	−0.07*	0.01*

Ta – air temperature, Ts – soil temperature, SM – soil moisture, H_2O – net H_2O exchange rate, correlations significant at $p<0.05$, * not significant suddenly increased and remained high, the soil CO_2 efflux rate dropped significantly. Thus, SM can cause increases or decreases in soil CO_2 rates in the short-term, but the influence of temperature is far greater across a seasonal time scale.

DISCUSSION

During our experiments we took measurements of CO_2 efflux, soil and air temperature and soil mosture. We performed experiments with only one tree species in order to avoid bias of data due to different tree species. Our results showed that CO_2 efflux rates changed according to diurnal and seasonal changes and that Ta and Ts showed the strongest correlation with soil CO_2 efflux. Ts was one of the most important environmental parameters and was responsible for the majority of the variability of efflux rates (Hu et al. 2011, Kopittke et al. 2012). However, Lingfei et al. (2020) found that efflux was more strongly correlated with Ta than with soil. Their study was conducted in tropical peatlands, so based on their results it is possible to infer that the key factors that regulate soil CO_2 efflux rates can vary by the climatic zone (Lingfei et al. 2020).

According to other studies, there may be other variables that affect the CO_2 efflux rate. The suggested variables include SM, photosynthesis, microbial growth, plant biological activities, and root respiration, depending on their size and distribution depth. Root respiration is important in terms of photosynthesis because it can determine a forest's ability to release carbon to the atmosphere. Depending on the tree species, climate, forest age, etc., root respiration can contribute to a total soil CO_2 efflux from 6% to 80%, and in some cases up to 90% (Yuste et al. 2005, Eberwein et al. 2015).

Diurnal CO_2 efflux variations

During the day, higher Ts and better light conditions can increase the rate of microbial metabolism, increasing CO_2 efflux rates (Hibbard et al. 2005). During diurnal studies, sudden temperature fluctuations or sudden changes in weather conditions also lead to fluctuations in soil CO_2 efflux. For example, during periods of rainfall, CO_2 efflux rates rapidly fluctuate because of the additional moisture added to the soil. Meanwhile, temperature conditions had a far less significant effect on seasonal studies. The results of other studies show that Ts regulates the respiration of plant roots and microbial activities related to C cycling in forest soil (Fierer et al. 2003, Yuste et al. 2005). During this study, it was observed that excess water slows or stops efflux completely when an excess of water in the soil reduces the diffusion of gases between the soil and atmosphere. This happened when measurements were taken during or right after heavy rainfall. Such data were not used because they were not reliable and did not show the real situation of CO_2 efflux.

During our observations, it was noticed that maximum Ta was reached earlier in the day than maximum Ts, and that CO_2 efflux had a stronger correlation with Ts and reached its maximum at a similar time of day. It is known that PAR (Photosynthetically Active Radiation) reaches its maximum earlier than Ts and at a similar time to Ta. Autotrophic respiration mainly responds to PAR and Ta, and when Ts reaches its maximum its combined respiration contributes to the maximum CO_2 efflux. Thus, there is a large time lag between maximum values of parameters. This time lag may create hysteresis between Ts and CO_2 accumulations.

The highest CO_2 efflux rate recorded during daytime was from 16:00-20:00, and the lowest was early in the morning, between 05:00-08:00. The same or similar results have been reported by different researchers in similar climate zones (Xu et al. 2011). The diurnal range normally varied between 0.4-1.8 μmol m^{-2} s^{-1}, or approximately 10-40% of its mean value depending on the year of study. Another study reported diurnal soil CO_2 efflux range values of approximately 1 μmol m^{-2} s^{-1} for a one-year study, suggesting that our research is valid and corresponds well with other similar studies (Xu et al. 2001). There are numerous reports from other studies suggesting that changes in weather conditions are responsible for significant drops in efflux rates. In this study, because of the rain, diurnal CO_2 efflux rates dropped by 3.43 μmol m^{-2} s^{-1}, i.e. by more than 50%. This confirms the assumption that moisture from rain has a significant impact on efflux rates in a diurnal context (Zhang et al. 2013, Hu et al. 2016).

Seasonal CO_2 efflux variations

In terms of the seasonal variation of CO_2 efflux, SM varied strongly because of temperature and precipitation changes. The average SM was between 10% and 15%, which is consistent with other studies conducted in Scots pine forests (Vanguelova et al. 2005). There is no substantial evidence to suggest that SM has a significant effect on soil CO_2 efflux rates. During our observations, it was noted that when Ts was very high and SM was very low, there could be a limitation placed on soil CO_2 efflux rates. In 2019 study, it was noticed that even when there was very high Ts and low or extremely low SM, CO_2 efflux could still be very high. This may mean that Ts is very important because when Ts is low and SM is low this dilutes the effect of Ts and efflux rates drop significantly. This effect appears when microbial activity is reduced because of a deficit of soil water and temperature. A similar effect has been reported in other studies where droughts occurred during measurements (Hibbard et al., Vanguelova et al. 2005), as a minimal level of SM is essential for microbial activity in decomposition processes. On the other hand, too much SM creates another limitation on CO_2 efflux. The CO_2 efflux may become separate from the temperature influence at certain SM levels, meaning CO_2 efflux may have no correlation with temperature when SM is too high. The optimum SM for soil CO_2 efflux is frequently found at intermediate levels, above or below which soil respiration is reduced. These findings suggest that Ts and SM are major determining factors of soil CO_2 efflux rates in Scots pine forest stands. These factors can also determine processes in the organic soil layer, where microbiological respiration and root respiration take place (Liang et al. 2004).

The calculation of the temperature coefficient suggested that increases in temperature had a positive effect on CO_2 efflux. In particular, Ts had a stronger effect than Ta did. The coefficient values varied from 1.1 to 3.5 and corresponded to ones determined for coniferous trees in other studies (Lloyd, Taylor 1994). It was noticed that when temperature dropped, at certain point Q_{10} values dropped as well, and Ta and Ts both had a similar impact on CO_2 efflux. This happens when seasons change, when warm periods are followed by cold periods – at this point both heterotrophic and autotrophic respiration decrease. Changes in SM can affect the composition and function of a soil microbial community due to differences in drought tolerance among taxonomic and functional groups of microorganisms (Lee 2018). In other studies, it has been observed that Q_{10} is inversely correlated with mean annual temperature, which corresponds with our observations (Meyer et al. 2018).

During our seasonal soil CO_2 efflux study, it was found that peak forest growth was related to peak efflux rates. In three different periods (2013, 2018, and 2019), peak efflux rates were observed in early to mid-June. This evidence supports the notion that at this time of year the microbial and photosynthetic activities are enhanced, and the same trends have been observed in previous studies (Eberwein et al. 2015, Lingfei et al. 2020).

Comparison diurnal and seasonal CO_2 efflux variations

All of the rates we recorded in this study were similar to ones in other experiments conducted worldwide in forest stands during both seasonal and diurnal variation studies (Xu, Qi 2001, Zhang et al. 2013). However, the variation patterns of soil CO_2 efflux rates in both diurnal and seasonal experiments were also similar to those in other studies on soil CO_2 efflux rates in areas that have clear seasonal climatic changes (Andersen et al. 2010, Zhang et al. 2013, Hu at al. 2016).

The diurnal rate values fluctuated within a certain range (2.78-6.17 μmol m^{-2} s^{-1}), while seasonal variations fluctuated more, but on average the CO_2 efflux rate was between 2 and 4 μmol m^{-2} s^{-1}. This corresponds well with most previous studies conducted in pine forests (Singh et al. 2003, Yuste et al. 2005,).

These results show that taking short- and long-term measurements with an open chamber system can provide continuous data on CO_2 efflux rates. Our results suggest that both diurnal and seasonal variation in efflux are strongly correlated with Ts at a depth of 10 cm. Abnormal CO_2 efflux fluctuations were observed when SM was either unusually low or high. SM has to be at moderate levels to sustain normal levels of efflux, to enable Ts to increase and enhance CO_2 efflux rates.

Further detail is required to examine all of the factors that control CO_2 efflux rates, which can include photosynthesis, activities of soil microorganisms, soil properties, roots, species composition, and anthropogenic factors. These factors must be investigated and more detailed data analyses completed to understand and predict changes in the cycle of C sinks in Scots pine forest stands.

CONCLUSIONS

The results obtained in this study indicate that diurnal and seasonal patterns of soil CO_2 efflux are strongly connected with temperature and SM. In particular, Ts at a depth of 10 cm had a higher impact on the efflux rate during our observations. The collected data suggest that SM has a weaker connection with the efflux rate but had an impact on Ts, dampening its effect on soil CO_2 efflux.

Ts alone does not explain all changes in soil CO_2 efflux. The changes in SM during seasons may have the potential to introduce factors that can affect the CO_2 efflux by changing diffusion rates and thermal parameters. These factors may explain the relationship between soil CO_2 efflux and Ts. Drought and excessive moisture greatly reduced efflux rates, and seasonal decreases in SM were correlated with decreases in the soil CO_2 efflux rate. It is expected that CO_2 efflux rates will rise due to an increasingly warm and dry climate. These findings contribute to a more comprehensive understanding of the factors that control the patterns of soil CO_2 efflux from pines forests over different temporal scales in Lithuania.

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