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Original article

Scots pine (*Pinus sylvestris* L.) roots and soil moisture did not affect soil thermal sensitivity

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ABSTRACT

Through their effects on microbial metabolism, temperature and moisture affect the rate of decomposition of soil organic matter. Plant roots play an important role in SOM mineralization and nutrient cycling. There are reports that rhizosphere soil exhibits higher sensitivity to temperature than root-free soil, and this can have implications for how soil CO_2 efflux may be affected in a warmer world. We tested the effects of 1-week incubation under different combinations of temperature (5, 15, 30 °C) and moisture (15, 50, 100% WHC) on the respiration rate of soil planted with Scots pine and of unplanted soil. Soil respiration in both soils was the highest at moderate moisture ($p < 0.0001$) and, increased with temperature ($p < 0.0001$). There was also marginally significant effect of soil kind on respiration rate ($p < 0.055$), but the significant interaction of temperature effect with soil kind effect, indicated, that soil respiration of planted soil was higher than unplanted soil only at 5 °C ($p < 0.05$). The soil kind effect was compared also as Q_{10} coefficients for respiration rate, showing the relative change in microbial activity with increased temperature. However, there was no difference in the thermal sensitivity of soil respiration between planted and unplanted soils ($p = 0.99$), irrespective of the level of soil moisture. These findings were similar to the latest studies and confirmed, that in various models, being useful tools in studying of soil carbon cycling, there is no need to distinguish between planted and unplanted soil as different soil carbon pools.

1. Introduction

Temperature and moisture are the most important factors affecting microbial metabolism and, in turn, the rate of decomposition of soil organic matter (SOM) [6,20]. The possible loss of SOM due to climate warming may increase the concentration of atmospheric CO_2 and result in a positive feedback effect on climate change [24]. Despite the numerous studies on the effect of temperature and humidity on SOM decomposition and carbon cycling in terrestrial ecosystems, there is still substantial uncertainty about whether climate change will shift ecosystems from being important carbon sinks to carbon sources [2,40,49]. The effect of biological variables on soil thermal sensitivity and the interaction of biotic and abiotic factors remain poorly understood.

The soil environment is highly heterogeneous, and plant roots are especially strong soil modifiers. The rhizosphere – the zone of influence of plant roots on soil – is a complicated belowground system composed of plant roots, soil and soil organisms, all closely dependent on each another. Plant roots play an important role in SOM mineralization and nutrient cycling. The relative contribution of roots and rhizosphere heterotrophs can range from 20% to 90% of the total field CO_2 flux from soils of the temperate climate zone [3,12,24]. Roots considerably alter local soil properties such as nutrient concentration, pH and redox potential, as well as gases such as O_2 and CO_2 exchange [18]. The most important phenomenon in the vicinity of roots is the stimulation of microbial activity by various organic exudates [17,27]. As compared to bulk soil, rhizosphere soil is characterized by higher biological activity, biomass and functional (metabolic) diversity, and by the distinctness of the taxonomic structure of the microbial community [1,14,27,41]. Bacterial communities of the rhizosphere soil differ from these of root-free soil, and some bacterial groups (e.g., *Pseudomonas*, *Flavobacterium*) are particularly stimulated in the rhizosphere by released root exudates [14]. Mycorrhizas, symbiotic relationships formed by some fungal species with plant roots are represented within rhizosphere microbiota also [45]. As such, in terms of the microbial ecology of soil, the rhizosphere can be referred as a 'hot spot' in bulk soil [34].

Because of these crucial differences, some authors suggest that the rhizosphere and root-free soil may differ in their sensitivity to

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such basic environmental factors as temperature and moisture. In view of global climate warming, these contradictory reports need to be carefully verified so that the global carbon cycle and carbon sequestration can be modelled more precisely [36]. Studies on the thermal sensitivity of soil CO_2 efflux and the interactions between temperature and other abiotic factors such as soil moisture have concentrated mostly on intact soil or root respiration [4,11]. Boone et al. [3] showed in a field experiment that the respiration rate, with a temperature range from 3 to 21 °C, increased more in roots and associated rhizosphere compared to root-free soil. They suggested that the thermal sensitivity of total soil respiration may depend on the relative contribution of roots and associated rhizosphere microbiota to the total soil CO_2 efflux. In contrast, Bååth and Wallander [1] found no difference between rhizosphere soil and root-free soil in sensitivity to temperature change in the range 5–22 °C. Also, using an experimental rhizocosms, Langley et al. [28] found that mycorrhiza presence influences the rate but not the temperature sensitivity of soil respiration. Temperature effect on total soil respiration is not always consistent, as factors such as soil water content, temporal patterns in root respiration and turnover of fine roots influence soil respiration [10]. The aim of this study was to test whether temperature and moisture affect soil respiration rate of the rhizosphere and root-free soil differently. It could be that only in some combinations of temperature rhizosphere soil will show higher respiration rate than bulk soil. The biological factors, as root presence may affect soil microbial response to temperature and moisture. The different response of rhizosphere and bulk soil may suggest necessity of distinguish of these carbon pools in soil when modelling the global carbon cycling. Comparing to other studies on soil thermal sensitivity of planted and unplanted soil, we applied additional environmental factor of high importance, the moisture.

2. Materials and methods

2.1. Soil sampling and incubation

Nursery-garden was founded in autumn 2004 in the field station of the State Forests in Jodłówka, southern Poland. The forest soil organic layer with composted green manure (70%:30% w:w) was used as the standard material for tree seedling cultivation. The green manure was composed with buckwheat, fodder sunflower, yellow lupine and phacelia. After mixing and sieving, the soil was put into 30 standard nursery containers (foil pots with holes in the bottom, volume ca 3 dm^3). Half of them were planted with one year-old Scots pine (*Pinus sylvestris* L.) seedlings (5 seedlings per container), and the rest were left without seedlings. In autumn 2005, when the root system was spread though the entire soil volume, all containers were brought to the laboratory for soil sampling. Unplanted soil was sampled from the pots with a steel sampler from five points of the container. Sampling of planted soil required gentle cutting of the foil pot, careful removal of the plants and separation of soil from the roots. Soil samples were then sieved (2 mm) and mixed to obtain two soil kinds: planted/rhizosphere soil (R) and unplanted/bulk soil (B). Before further processing and analyses, the R and B soils were kept for two weeks in the laboratory at 4 °C to lessen the effect of soil sampling.

Afterwards, the R and B soil samples were incubated for one week in three climate chambers at three temperatures (5 °C, 15 °C, 30 °C) and three soil moisture levels (15%, 50%, 100% water holding capacity, WHC), giving altogether nine treatments in a full-factorial design. There were three replicates for each combination of soil kind, temperature and moisture treatment, for a total of 54 samples. Sample moisture was adjusted with deionised water on a daily basis. The incubation time was intended to capture root effect and minimise changes in microbial communities caused by prolonged soil storage, especially at higher temperatures [38], which may result in the degradation of carbon sources such as root exudates, dead microbial cells and fine roots.

2.2. Chemical analyses

The dry weight (dwt) of soil was determined by drying subsamples at 105 °C for 24 h. Organic matter (OM) content was determined as loss on ignition at 550 °C for 24 h. Water holding capacity (WHC) was measured by a standard gravimetric method using 50 g subsamples. Soil pH was measured in water and in KCl after 1 h shaking of 5 g soil in 50 cm^3 deionised water (pH_{H_2O}) or 1.5 g soil in 15 cm^3 1 M KCl (pH_{KCl}). Total concentrations of Ca, Mg, Mn, K and Na were measured after wet digestion of 0.5 g fine-ground subsamples in 10 ml concentrated boiling HNO_3. Ca, K and Na were measured by emission flame spectrometry (JENWAY, PFP 7), and Mg and Mn by flame atomic absorption spectrometry (PERKIN-ELMER, AAnalyst 800). Total C and total N content in fine-ground subsamples was analysed with a CHNS analyser (Vario EL III, Elemental Analyser GmbH). The analysis of each element occurred in replicates of three ($n = 3$) on the R soil and of two replicates ($n = 2$) on the B soil; there were also five replicates ($n = 5$) on soil before the initiation of the whole experiment.

2.3. Respiration rate and Q_{10} coefficients

Total microbial activity of R and B soil was measured as the respiration rate in combinations of three temperatures and three soil moisture levels as described above. The respiration rate was measured in equivalents of 10 g soil dwt by trapping CO_2 in 5 ml 0.2 M NaOH in airtight glass jars. With 27 combination of experimental treatments and 3 replications for each experimental treatment, the total number of samples (and jars) was 54. Also, five empty jars randomly distributed among the others were used as blanks. After the samples were incubated in closed jars for 12–48 h (depending on the incubation temperature and soil moisture), 2 ml $BaCl_2$ was added to the NaOH solution, and the excess sodium hydroxide was titrated with 0.1 M HCl in the presence of phenolphthalein as indicator. Between measurements the jars were left open. The respiration rate for each sample was expressed as mM CO_2 per kg organic matter per 24 h. At the end of incubation the organic matter content was determined for each soil sample for accurate estimation of the respiration rate.

After the respiration rate was measured at three different temperatures, the Q_{10} temperature coefficients were calculated: $Q_{10}L$ (Low; based on the respiration rates measured at 5 °C and 15 °C) and $Q_{10}H$ (High; based on the respiration rate measured at 15 °C and 30 °C). The Q_{10} coefficients were calculated for samples of R and B soil and each moisture treatment, using the average respiration rates (R) at the two consecutive temperatures (T) [13]:

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

2.4. Statistical analyses

For soil respiration rate, a three-way ANOVA was used to test differences between soil kind (R, B), significance of the effects temperature (5 °C, 15 °C, 30 °C) and moisture (15%, 50%, 100% WHC), and their interactions. For the Q_{10} values of the soil respiration rate, a three-way ANOVA was used to test differences between soil kind (R, B), temperature range (L, H), moisture (15%, 50%, 100% WHC) and their interactions. Right- or left-skewed data were transformed to