Effect of shungite application on the temperature sensitivity of *Allium cepa* respiration under two soil water regime

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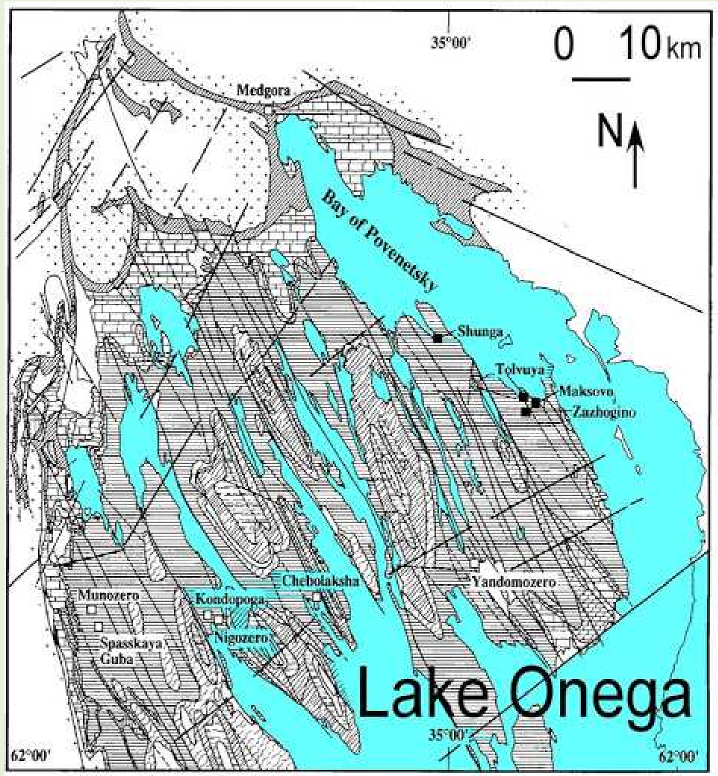
Introduction

The possibility of using rock powders as an alternative source of nutrients for agriculture practice has been widely discussed. Shungite rocks formed mainly on a silicate basis are carbon-bearing sedimentary-volcanic rocks found in the Onega lake area.



The carbonaceous matter characterized by globular fullerene-like molecular structure is one of the main components of the shungite. Along with inorganic carbon, some macro- and micronutrients, such as Si, K, Ca, Mg, Na, Cu, and others were found in the shungite rocks. Since the most of nutrient elements are prevalent soil elements beneficially affect the physiological state of plants, maintaining adequate plant nutritional status may improve the physiological resistance of plants under stress situations, including stress temperatures and soil water deficit .

The aim of this study

Plant respiration is a temperature-sensitive process with the temperature sensitivity being referred to as the temperature coefficient (Q_{10}), defined as a proportional change in respiration rate per 10°C change in temperature. The Alt and Cyt pathways of mitochondrial respiration have been shown to differ in their sensitivities to short-term changes in temperature. It was proposed that the Alt pathway may maintain mitochondrial electron transport and protect against harmful reactive O_2 generation in the cold due to this pathway being less temperature sensitive (lower Q_{10}) than Cyt pathway. However, some studies found little difference in the Q_{10} values between the Alt and Cyt pathways or more sensitive Alt than Cyt pathway. The shift of the temperature sensitivity of these respiratory pathways under changed conditions, for example, soil water or nutrient availability, can alter the partitioning between the pathways, and, consequently, plant resistance to temperature stress.

Our study investigated the effect of short-term changes in temperature on respiration in intact tissues of onion leaves. We examined whether the Q_{10} values of SHAM-resistant and SHAM-sensitive respiration differs and how shungite application to soil affects the temperature sensitivity of both SHAM-resistant and SHAM-sensitive respiratory pathways. Moreover, we established the extent to which the shungite dependence of SHAM-resistant and SHAM-sensitive pathways is affected by a change in the soil water availability.

Materials and Methods

The soil used in this study was collected from the 0-30cm topsoil layer of an Umbric Podzols characterized by low natural fertility, thin layer, low content of humus, as well as, low pH (4–5). Shungite was taken from the mineral Zazhogino deposit (Karelia, Russia). The entire volume of the dry soil was divided into four parts and mixed with shungite powder.

Four concentrations of shungite powder were used in this experiment: 0, 5, 10, and 20 g of shungite per 1 kg of dry soil, designated as 0S, 5S, 10S, and 20S, respectively. Before seed sowing, all soils were incubated under 21-23°C and 70-80% of the maximum soil water holding capacity for 90 days.

Materials and Methods

Plant growth conditions

The soil substrates were parked into plastic pots (12 cm wide, 16 cm height). Before sowing, uniform seeds of onion (*Allium cepa* L., var. Sturon) were imbibed in water for 3 h and sown with six seeds per pot. All pots were subjected to a controlled climate chamber (Vötsch BioLine, Balingen, Germany) with conditions of 23/20°C day/night temperature, 70% relative air humidity, 16-h photoperiod, 300 μmol m^{-2} s^{-1} of photosynthetic photon flux density. All pots were maintained well-watered for one week until seedlings were thinned to three seedlings per pot.

One week after sowing the pots of the 0S, 5S, and 10S treatments were randomly divided into two blocs and two watering treatments were applied: well watering (WW) and drying-wetting cycles (DW).

Materials and Methods

Total and SHAM-resistant respiration measurement

Total respiration and SHAM-resistant respiration of leaves and roots were measured using a Clark-type oxygen electrode (Oxygraph Plus, Hansatech, Norfolk, UK) at 23°C. A leaf sample was harvested, cut into small pieces, and suspended in 2 mL of air-saturated 100 mM Hepes buffer (pH 7.5) in the reaction vessel of the electrode unit. The O_2 uptake rate was measured in the presence of salicylhydroxamic (SHAM) acid, an agent commonly used as an inhibitor of alternative pathway respiration (Alt), or in the absence of the SHAM. The rate of oxygen uptake by plant samples in a SHAM-free buffer solution was defined as total respiration (V_t); O_2 uptake rate in the SHAM-containing buffer was defined as SHAM-resistant respiration ($V_{SHAM\text{-}res}$), and the difference between V_t and $V_{SHAM\text{-}res}$ was defined as SHAM-sensitive respiration ($V_{SHAM\text{-}sens}$). By neglecting the influence of SHAM on Cyt pathway activity, the contribution of $V_{SHAM\text{-}sens}$ to the V_t rate (%) was calculated as $V_{SHAM\text{-}sens}/V_t$ ratio.

Materials and Methods

Temperature response of O_2 uptake rates

To determine a temperature response of V_t, $V_{SHAM\text{-}res}$, and $V_{SHAM\text{-}res}$ respiratory pathways of onion leaves of O_2 uptake rates were measured at a buffer solution temperature of 13, 23, and 33°C. The required temperature was attained by connecting the reaction vessel with a water-bath thermostat (VEB MLW Prufgerate-Werk, GDR). The Clark-type oxygen electrode calibration was carried out at each measurement temperature.

The temperature sensitivity of O_2 uptake rates was evaluated using the temperature coefficient (Q_{10}) that shows the proportional change in a respiration rate with a 10°C increase in temperature. The Q_{10} values were determined by approximating the plots of respiration rates at different temperatures with a power function.

Results

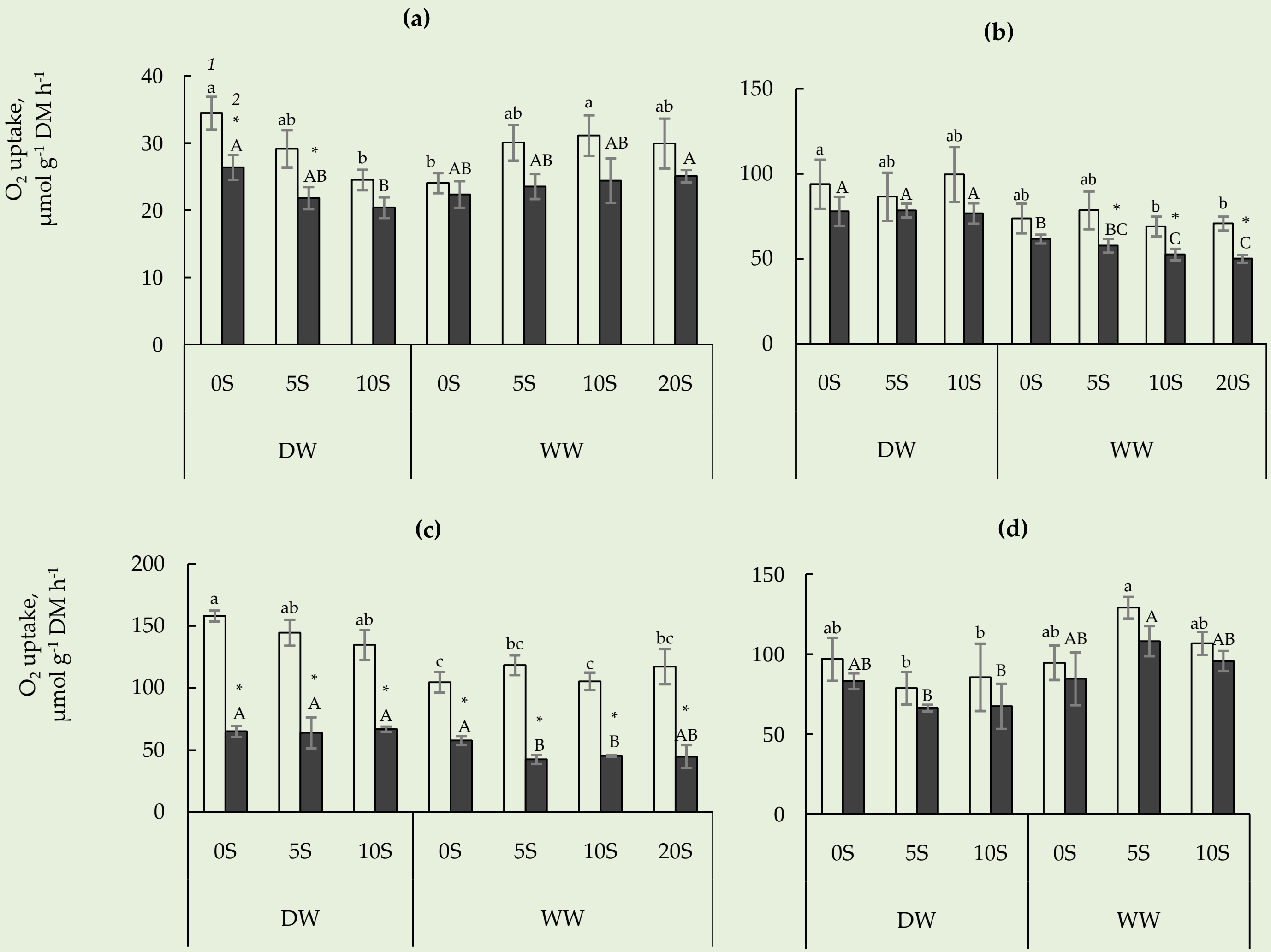


Figure 1. Total respiration (*1*) and SHAM-resistant respiration (*2*) for onion leaves (**a-c**) and roots (**d**) grown on the Umbric Podzols with shungite concentration of 0 (0S), 5 (5S), 10 (10S), and 20 (20S) g kg^{-1} under drying-wetting (DW) or well watering (WW) regime. During the measurements, the temperature was kept at 13 (**a**), 23 (**b**), or 33 (**c**) °C. Different letters indicate significant differences. * indicates significant differences between V_t and $V_{SHAM\text{-}res}$ at $P < 0.05$.

Results

Total and SHAM-resistant respiration

For the 0S seedlings, a significant impact of soil water deficit on total (V_t), but not SHAM-resistant ($V_{SHAM-res}$), respiration was found under low (13°C) and high (33°C) measurement temperature (Figure 1a, c). The leaf V_t rate was higher in 0S seedlings grown under DW, than WW condition. On the contrary, at 23°C, no significant differences in the V_t rates of leaves and roots were found between 0S seedlings grown under DW and WW regimes, but $V_{SHAM-res}$ was higher in DW than WW leaves (Figure 1b). According to the two-way ANOVA, the $V_{SHAM-res}$ rate of both leaves and roots was significantly affected by soil water availability, but the effect of shungite application was not significant for both V_t and $V_{SHAM-res}$ rates at all measurement temperatures (Table 1). However, for certain conditions of temperature and soil water availability, this effect was enough significant. So, shungite application decreased leaf V_t and $V_{SHAM-res}$ under DW and increased V_t under WW regime at 13°C (Figure 1a). Moreover, under the WW regime and at the temperatures of 23 and 33°C, seedlings grown on the soil containing shungite had lower $V_{SHAM-res}$ values than 0S seedlings (Figure 1b, c).

Results

Table 1. Statistical results (*P*-value) of two-way ANOVA for the parameters shown in Figure 1 and 2.

Variables	Treatment factor, interaction		
	Shungite	**Water regime**	**Shungite + Water regime**
Leaves			
13°C			
V_t	0.233ns	0.194ns	0.052ns
$V_{SHAM\text{-}res}$	0.310ns	0.192ns	0.118ns
$V_{SHAM\text{-}sen}/V_t$	<0.001***	0.702ns	0.047*
23°C			
V_t	0.606ns	0.093ns	0.420ns
$V_{SHAM\text{-}res}$	0.231ns	<0.001***	0.582ns
$V_{SHAM\text{-}sen}/V_t$	0.049*	0.039*	0.030*
33°C			
V_t	0.551ns	0.093ns	0.394ns
$V_{SHAM\text{-}res}$	0.231ns	<0.001***	0.440ns
$V_{SHAM\text{-}sen}/V_t$	<0.001***	<0.001***	0.621ns
Roots			
23°C			
V_t	0.788ns	0.042*	0.161ns
$V_{SHAM\text{-}res}$	0.902ns	0.022*	0.216ns
$V_{SHAM\text{-}sen}/V_t$	0.405ns	0.108ns	0.621ns

V_t, total respiration; $V_{SHAM\text{-}res}$, SHAM-resistant respiratory pathway; $V_{SHAM\text{-}sen}$, SHAM-sensitive respiratory pathway

Results

Ratio of SHAM-sensitive to total respiration

The increase of respiration sensitivity to the SHAM reflects an increase in the contribution of Alt respiratory pathway to total respiration. With the increase of the measurement temperature, the $V_{\text{SHAM-sens}}/V_{\text{t}}$ ratio tended to increase (Figure 2). For the 0S leaves, $V_{\text{SHAM-sens}}/V_{\text{t}}$ values were higher in DW than WW seedlings regardless of the measurement temperature.

In contrast to the roots, for the leaves, the two-way ANOVA revealed a significant effect of both shungite application and soil water availability and their interaction on the $V_{\text{SHAM-sen}}/V_{\text{t}}$ ratio (Table 1). In contrast to DW cycle, shungite application significantly increased the leaf $V_{\text{SHAM-sen}}/V_{\text{t}}$ values, for the seedlings grown under WW regime at all measurement temperature regardless of soil shungite content (Figure 2).

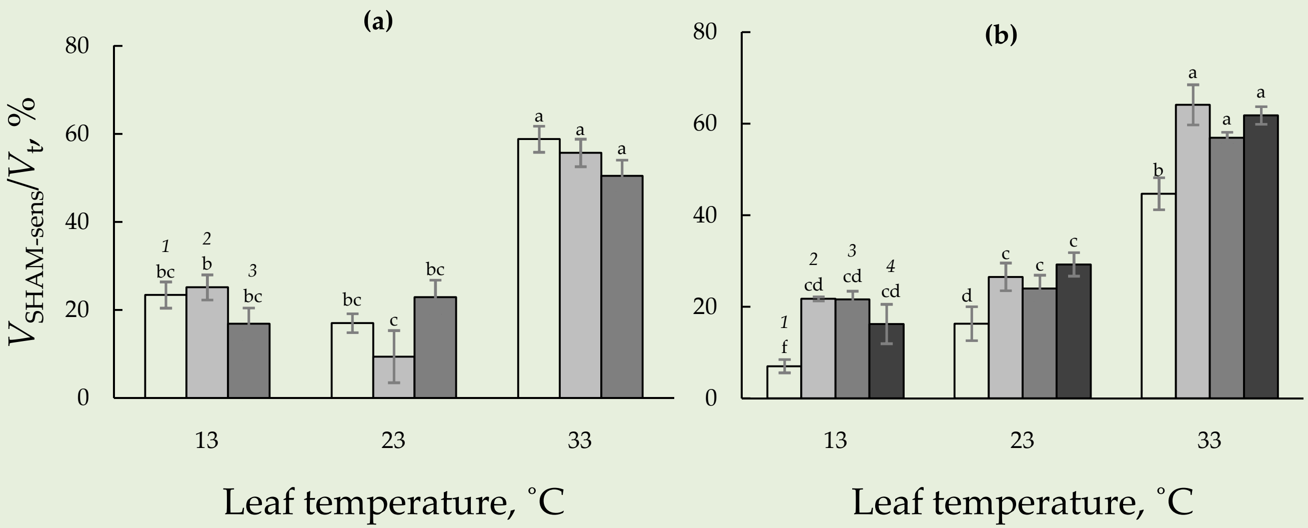


Figure 2. The $V_{\text{SHAM-sen}}/V_{\text{t}}$ ratio for leaf respiration of onion seedlings, grown on the Umbric Podzols with shungite concentration of 0 (0S), 5 (5S), 10 (10S), and 20 (20S) g kg^{-1} under drying-wetting (**a**) or well watering (**b**) regime.

Results

Respiratory coefficient (Q_{10})

The Q_{10} values of leaf V_t and $V_{SHAM\text{-}res}$ did not differ significantly between the 0S seedlings grown under DW and WW regimes, but Q_{10} of $V_{SHAM\text{-}sens}$ decreased strongly following the decrease in soil water availability (Figure 3). Regardless of shungite treatment and soil water regime, the Q_{10} values were higher for the $V_{SHAM\text{-}sens}$, than V_t and $V_{SHAM\text{-}res}$ rates. The opposite effect of shungite application on respiratory coefficient was found for seedlings grown under DW and WW regimes. While under DW condition, shungite application increased Q_{10} of both $V_{SHAM\text{-}res}$ and $V_{SHAM\text{-}sens}$, increasing V_t, under the WW regime, shungite significantly decreased respiratory coefficient of both respiratory pathways.

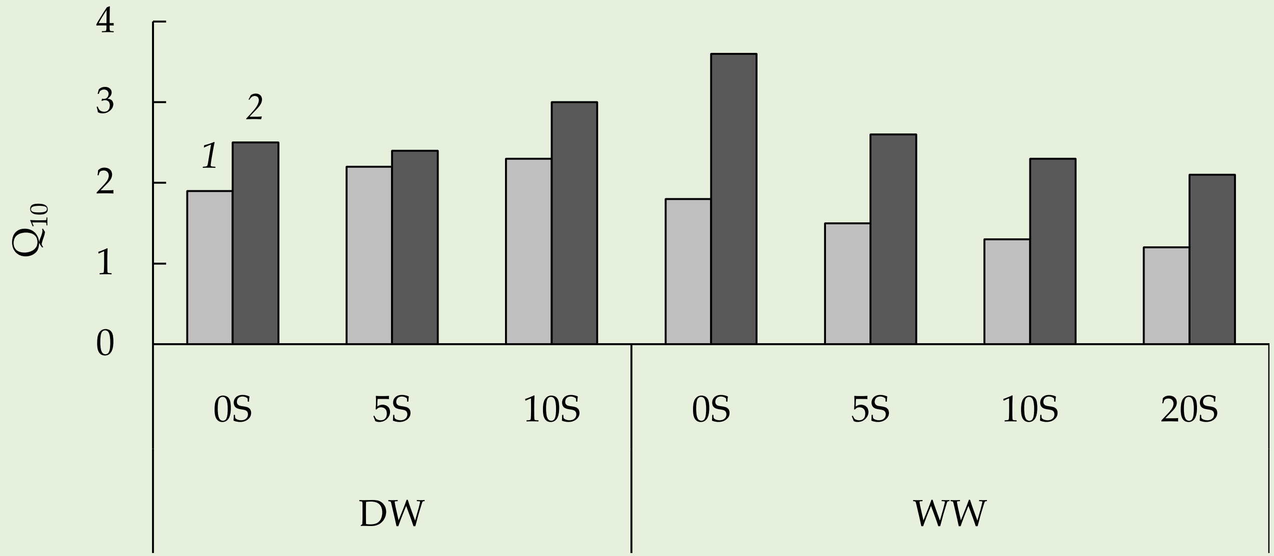


Figure 3. Temperature sensitivity (Q_{10}) of the total (1), SHAM-resistant (2) and SHAM-sensitive (3) leaf respiration of onion seedlings growing on the Umbric Podzols with shungite concentration of 0 (0S), 5 (5S), 10 (10S), and 20 (20S) g kg^{-1} under drying-wetting (DW) or well-watered (WW) regime.

Conclusions

The data demonstrate that both SHAM-resistant and SHAM-sensitive respiratory pathways of *A. cepa* leaves and their sensitivity to short-term temperature change can be dynamic when plants are subjected to the contrasting conditions of soil water availability or shungite content. For plants grown without shungite, water deficit decreased the Q_{10} values of SHAM-sensitive, but not SHAM-resistant respiration. The response of the temperature sensitivity of the pathways to shungite application depends on the water availability. The shungite-related decrease of both SHAM-resistant and SHAM-sensitive pathways may play an important role in enhancing the resistance of plant respiration to the temperature drop.

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