Effects of CO₂ Enrichment on the Responses of Legume-Rhizobia Symbiosis to Elevated Soil Temperature

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Introduction

The Intergovernmental Panel on Climate Change (2014) has predicted that the global mean surface temperature may rise 0.3°C - 0.7°C by 2035, a continuation of the gradual warming caused by the release of carbon dioxide and other greenhouse gases into the atmosphere. Rising temperature and CO₂ concentration, both separately and together, can strongly affect the growth of plants by changing photosynthesis rates, nutrient and water availability, and other key aspects of the growth environment (Lira et al, 2005; DeLucia et al., 1999; Phillips et al., 1976). It is therefore important to continue studying plant responses to temperature and CO₂ fluctuations as we try to prepare for future climate change. We focus on legumes because they are important food crops that are planted alternatively with other crops to enrich the soil via a symbiotic relationship with nitrogen-fixing rhizobial bacteria. Previous studies investigated the effect of either only temperature or CO₂ on the legume-rhizobia symbiosis, and few have looked into the interaction of these two factors. The factorial experiments that have been conducted mostly discuss the response of the plant and fail to provide a holistic understanding on the symbiosis system (Aranjuelo et al., 2005; Wang et al., 2011).

Regarding CO₂ as a single factor, various studies found that CO₂ enrichment increases the rate of photosynthesis and thus increases plants’ production and root proportion (DeLucia et al., 1999; Schaffer, Whiley & Searle, 1999; Rogers et al., 1996). As atmospheric CO₂ levels continue to increase and C3 plants produce more carbon photosynthate, bacteria growth will be stimulated since the rhizobia acquire carbon from the plants. However, the long-term effect of CO₂ enrichment on the mutualistic relationship remains unclear, since other studies pointed out the uneven N-partitioning between plant-microbial symbiosis will alter their interaction in favor of nitrogen intake of plants and suppress microbial decomposition (Hu et al., 2001). Some researchers also found that over longer periods (months or years rather than days), plants, including legumes, down-regulate their rates of photosynthesis in response to elevated CO₂ (Aranjuelo et al., 2005).

Another factor, soil temperature, is likely to have various effects on legume-rhizobium symbiosis. It is known that changes in soil temperature affect temperature-dependent processes such as belowground respiration, decomposition, and root nodulation. Increases in soil temperature result in higher rates of soil respiration (Lloyd & Taylor, 1994; Peterjohn et al., 1994), decomposition (Swift et al., 1979) and higher nitrogen mineralization (Peterjohn et al., 1994). However, how root nodulation will respond to warmer soil temperature is inconclusive. According to Lira et al. (2004), increased root temperature accelerates nodulation and nodule growth rate for bean (Phaseolus vulgaris L.), but effects are not consistent with crops originated in different climate zones. Other studies revealed that although nodule number increases in higher root temperature, high temperature induces detrimental effects on later development of the nodules by reducing nodule size and nitrogen fixation, which subsequently decreases plant growth. These effects also varied among species of both hosts and strains (Piha & Munns, 1987; Gibson, 1963; Gibson, 1971). Furthermore, soil temperature is an important factor that determines crop management. For example, according to Climate Change Impacts on Iowa (2011), fall application of nitrogen is usually conducted when soil temperature drops below 50°F. Higher temperature results in soil fertilization delay, leading to declined crop yield.

It is still unclear how the early growth of legume-rhizobium symbiosis will respond to new interactions between soil temperature and CO₂. According to Wang et al. (2012), higher CO₂ concentration increases net photosynthesis of legumes...
at heat stressed environments.

Since the results are far from comprehensive in understanding how legume-rhizobium symbiosis will be affected by simultaneous changes in temperature and CO2, we seek to explore the interaction between the two factors. In our study, we investigated three questions: (1) how will elevated CO2 affect legume-rhizobium symbiosis, (2) how will elevated soil temperature affect legume-rhizobium symbiosis, and (3) how will the interaction of elevated temperature and CO2 enrichment affect the symbiosis. Since CO2 and soil temperature response experiments on legume-rhizobia symbiosis are species-specific (West et al., 2005; Piha & Munns, 1987), we chose to study P. sativum, a model species of the Fabaceae family, and also an important crop cultivated all over the world. Since early growth of plants is crucial for their performances in later growth, such as responses to environmental stress (Grant et al., 2001; Aspinall, Nicholls & May, 1964), we focused our study on the first 4 weeks after germination. We hypothesized that (1) elevated CO2 levels enhance the legume-rhizobia mutualism through increased rate of photosynthesis and nodulation in its early development. Since the primary production of a plant is stimulated by CO2 enrichment, the growth of bacteria will increase and supply the plants with more nitrogen, thus forming a positive loop. We predicted that both the total biomass (g) and the root proportion (percentage of root biomass to total biomass, %) of legumes growing in elevated CO2, ambient soil temperature will be larger than those in ambient CO2, ambient soil temperature; the nodule abundance will be larger, and C:N ratio in plants, an indicator of nitrogen limitation, will decrease. (2) Elevated soil temperatures inhibit the mutualism through increasing root respiration in plants and reducing nodule nitrogen fixation (Lloyd & Taylor, 1994; Peterjohn et al., 1994). While high soil temperature initially stimulates nodulation (Lira et al., 2004), it will retard nitrogen fixation in later development of the symbiosis (Gibson, 1963; Gibson, 1971). Thus, we predicted that the total biomass and root proportion of the legumes growing in elevated CO2, elevated temperature will be larger than those in ambient CO2, elevated temperature; nodule abundance will increase and C:N ratio will decrease.

Methods

A factorial experiment was conducted with 4 groups of P. sativum (sample size of five) growing in (1) 400ppm, 25°C, the control group; (2) 400ppm, 30°C; (3) 800ppm, 25°C, (4) 800ppm, 30°C. Twenty P. sativum seeds were planted with inoculants in a 20-cell flat. After all the seeds germinated, 10 plants were moved to a chamber with CO2 controlled at 400ppm and air temperature maintained at 25°C, where the soil temperature of 5 cells was elevated to 30°C with a heat mat. The other 10 cells were placed in another chamber with CO2 levels at 800ppm and air temperature at 25°C, and similarly the soil temperature of 5 cells was elevated to 30°C with a heat mat. All the seedlings were supplied with constant light and 30ml of water on a daily basis. After four weeks of germination, we dried the plant tissues at 60°C for 24 hours, measured total plant biomass and root proportion. Afterwards, we used a flash combustion elemental CN analyzer to measure C:N. We used ANOVA to determine the effects of elevated soil temperature and CO2 on total biomass, root proportion, C:N ratio, and nodule abundance.

Result

Total Biomass

P. sativum grown in elevated CO2/ambient soil temperature had significantly higher total biomass than those grown in ambient CO2/ambient soil temperature (Fig. 1). There was no significant interaction between soil temperature and CO2 concentration on total biomass (Fig.1).
temperature, \( p = 0.010 \) for effects of CO2, and \( p = 0.790 \) for CO2 * temperature.

**C:N Ratio**

Pisum sativum grown in elevated CO2/ ambient soil temperature had significantly lower C:N ratio than those in ambient CO2/ambient soil temperature (Fig. 2). There was no significant interaction between soil temperature and CO2 concentration on C:N ratio of Pisum sativum grown in elevated CO2/elevated soil temperature (Fig.2).

![Fig. 2 Interaction plot for C:N ratio of Pisum sativum grown at 400 ppm and 25°C, 400 ppm and 30°C, 800 ppm and 25°C, 800 ppm and 30°C after 4 weeks. N=5 for each group. \( p = 0.548 \) for effects of soil temperature, \( p < 0.001 \) for effects of CO2, and \( p = 0.9731 \) for CO2 * temperature.](image)

**Root Proportion**

The elevated CO2/elevated soil temperature group showed significantly smaller root proportion relative to the ambient group (Fig. 3). Soil temperature and CO2 levels had no effects on root proportion.

![Fig. 3 Interval plot for root proportion Pisum sativum grown at 400 ppm and 25°C, 400 ppm and 30°C, 800 ppm and 25°C, and 800 ppm and 30°C after 4 weeks. N=5 for each group. \( p = 0.837 \) for effects of soil temperature, \( p = 0.283 \) for effects of CO2, and \( p = 0.048 \) for CO2 * temperature.](image)

**Significance**

Consistent with our hypothesis, elevation of CO2 benefited the legume-rhizobia symbiosis. Legumes in elevated CO2/ambient soil temperature had larger biomasses and lower C:N ratios, indicating a positive effect of CO2 enrichment on legume’s growth. This is explained by the fact that removal of carbon limitation and increase in water use efficiency promote photosynthetic rate in legume species (Cernusak et al., 2011), which not only increases plant’s biomass but also provides more photosynthate for the growth of bacteria. This is consistent with past findings that CO2 enrichment enhances nitrogen reduction short-term (Phillips et al., 1976). Therefore, the bacteria supply the plants with more nitrogen, forming a positive loop.

On the other hand, Pisum sativum grown at higher soil temperature had lower total biomass. We also observed much less nodulation than expected for the elevated soil temperature groups, which may indicate that elevated temperatures delayed or inhibited nodule development. The delay of nodule development most likely results from unsuccessful formation and hastened degeneration of bacteroid tissue under high root temperatures (Gibson 1971). Moreover, the elevated soil temperatures could also increase root respiration and reduce the amount of photosynthate available for bacterial growth (Lloyd & Taylor, 1994).

Contrary to our hypothesis, the results suggested that the interaction between elevated CO2 and elevated temperature did not benefit the symbiosis in the short-term. The group with both factors elevated showed lower total biomass and smaller root proportion, suggesting that the effects of CO2 did not counteract negative effects of high soil temperature on the symbiosis.

Corresponding with other studies on legume species, nodulation and nitrogen fixation of Pisum sativum are sensitive to change in CO2 levels and soil temperature (Piha & Munns, 1987; Lira et al., 2005; Philips et al., 1974). We speculate that legume-rhizobia symbiosis is sensitive to variation in soil temperature during nodule development and performance. We believe this holds important implications for crop yield as global warming progresses.

These results are relevant to crop management on soil fertilization as CO2 levels continue to increase,
raising temperature and impacting climate patterns. We not only demonstrated the detrimental effects of higher soil temperature on the growth of legume-rhizobia symbiosis, but we also showed that elevated CO2 did not offset higher soil temperature. Thus, it may be too optimistic to believe that higher CO2 levels will stimulate photosynthetic processes as a result of climate change. We suggest further studies on the long-term effects of the interaction between CO2 levels and soil temperature, as well as the ways that other factors like precipitation and air temperature affect the legume-rhizobia symbiosis.

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