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Effects of Elevated CO_2 and N Enrichment on Wetland Vegetation and Soil Microbial Community

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1. Introduction

Since the industrial revolution, the CO_2 concentration in the atmosphere has increased from about 275 ppm to the current level of 365 ppm. This concentration is expected to continue to increase, doubling from the current level during the next century. One consequence of an increased concentration of atmospheric CO_2 is an increase in plant biomass production due to higher net C assimilation. The influence of elevated atmospheric concentrations of CO_2 on plants is not limited to aboveground plant growth, but belowground processes are also likely to be affected. An increase in root biomass, total rhizodeposition, and changes in chemical composition of plant tissues and root exudates may occur under elevated CO_2 levels. These alterations are likely to affect function and structure of soil microbial community. In addition, nitrogen (N) enrichment is another environmental problem at a regional scale. N originated from anthropogenic activities has increased dramatically, which results in increase in atmospheric N deposition as well as N enrichment in river and estuaries. As CO_2 assimilation is closely related to N availability, interaction of elevated CO_2 and N enrichment is of great importance to elucidate possible trajectories of ecosystems impacted. Unlike the terrestrial ecosystem studies, however, relatively less efforts have been made to elucidate possible impacts of elevated CO_2 and N enrichment on wetland ecosystems. Although wetland ecosystems including peat-forming wetland cover only 2-6 % of global land surface, they play a pivotal role in global biogeochemical cycles. As such, this study aims to determine effects of elevated CO_2 and N addition on wetland plant and microbes through a laboratory-based experiment.

2. Materials and Methods

Wetland plants (*S. lacustris, J. effuses, M. sacchariflorus, P. japonica, T. latifolia, P. communis*, and *Z. latifolia*) were grown in pots under two levels of CO_2 (370 ppm or 740 ppm) and two levels of nitrogen (0 mg/L-N or 8.8 mg/L-N) within growth chambers for 110 days. At every 3 weeks, shoot length and extracellular enzyme activities in pore-water was measured. Enzyme activities were determined using methylumbelliferyl substrates. After 110 days, plant and soil samples were destructively collected to determine dry weight of shoots and roots, enzyme activities of soil, and bacterial community structure in the soil. In order to understand the effect of elevated CO_2 on the

bacterial community structure in wetland soil ecosystem, two types of molecular approaches were employed. For analyzing denitrifying bacterial community structure, functional genes such as nitrite reductase genes (*nirS*) were PCR-amplified and PCR products were cloned and screened by restriction fragment length polymorphism (RFLP). To explore the dynamics of the community composition of bacteria, the rRNA intergenic spacer analysis (RISA) targeting the 16S-23S intergenic spacer region from the bacterial rRNA operon were used. Soil samples planted with *T. latifolia*, *S. lacustris*, and *J. effusus*, were considered for this microbial analysis.

3. Results and Discussion

1) Plant biomass

Aboveground wetland plant biomass (including dead parts) increased in all pots during the experiment except *M. sacchariflorus*. Exposure to elevated CO_2 had a negative effect on shoot biomass growth in 7 species plants, resulting in a 6.8% decrease in total shoot dry weight on the average. N enrichment resulted in a larger amount of shoot biomass (33.6% on the average), but the differences were not significant (P = 0.830, 0.382, 2×2 ANOVA) due to large variability among 7 species of plants. In general, responses of plants to elevated CO_2 and N enrichment were species-specific. For example, above ground response to increased CO_2 concentration was positive for *S. lacustris* and *J. effuses*, but negative for *M. sacchariflorus*, *P. japonica*, *T. latifolia*, *P. communis*, and *Z. latifolia*. None of elevated CO_2 response is statistically significant, but N treatment effects were significant for *P. japonica*, *T. latifolia*, and *Z. latifolia*.

Shoot height of wetland plants increased continually in all treatments during the experiment, but a net height increase occurred mainly until 90 days. Throughout the experiment, the relative increase was the greatest under elevated CO_2 combined with high N supply ($P < 0.05$, one-way ANOVA). In the ambient CO_2 and low N treatment, the growth of shoot height was faster until 40 days of the experiment, but it almost stopped afterward. Elevated CO_2 appeared to affect wetland plant growth only with high N addition.

Belowground biomass of 7 species plants has been measured after 110 day of exposure by drying at 70 °C to constant weight. The relative change of belowground response to increased CO_2 and high nitrogen concentration ranged from -16.4% to 6.4% and -10.2% to 16.2%, respectively. However, none of these responses are statistically significant. Exposure to elevated CO_2 and nitrogen enrichment had a pronounced effect on root-shoot partitioning. The belowground biomass relative to aboveground biomass under elevated CO_2 was a 2.7 in plants grown under elevated CO_2 concentration comparing with that of 2.2 where exposed to ambient air. In contrast to response of plants exposure to elevated CO_2 concentration, addition of nitrogen led to decrease in the degree of ratio from 2.9 to 2.0 significantly ($P < 0.001$, two-way ANOVA).

2) Enzyme activities in pore-water

Around every 3 weeks, soil pore-water was collected from each pot and four kinds of enzyme activities were measured using methylumbelliferyl substrates. All enzyme activity in pore-water

increased continuously until 60 days of the experiment, but after 90 days, all enzyme activity decreased due to decline of plant growth. Phosphatase, arylsulfatase, β-glucosidase and *N*-acetylglucosaminidase activities were ranged 2.21-59.79, 0.08-0.7, 0.31-1.96, and 0.14-2.00 nmol ml^{-1} $hour^{-1}$, respectively. Phosphatase activity was the highest followed by *N*-acetylglucosaminidase, *β*-glucosidase, and arylsulfatase in all treatments. Significant differences in all enzyme activities were detected between treatments, and enzyme activities might be expected to change following elevated CO_2 and nitrogen deposition. However, there were no general trends under CO_2 and nitrogen treatments. The addition of nitrogen caused the phosphatase activity to increase significantly in pore-water except the first sampling occasion through the experiment ($P <$ 0.1). Arylsulfatase activity was also increased by high nitrogen treatment. β-glucosidase and N-acetylglucosaminidase activities in the pot under high nitrogen were higher than other pots. However the difference was not significant except N-acetylglucosaminidase activity at the third sampling occasion.

3) Soil microbial community

NirS gene fragments were amplified in all soil samples. A total of 62 clones (from the soils incubated under elevated CO_2) and 65 clones (from the soils incubated under ambient CO_2) were screened by RFLP. The species richness evaluated by the number of distinct RFLP patterns was 27 and 29 in the elevated CO_2 treatment and the ambient treatment, respectively. For *nirS* clones from the soils exposed elevated CO_2, 82% of all clones were composed of two dominant groups, while 53% of the clones from the ambient soils were composed of the same groups. Among all the distinct patterns identified in the two soils, only three patterns were found in both soils. For RISA, different fingerprints and consequently different genetic structures were observed between the elevated CO_2 and the ambient CO_2 samples. Overall, the data in this study showed that the denitrifying communities in the wetland soil ecosystem have many members and that the richness of denitrifying bacterial community was not significantly affected by CO_2 treatment.