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Impact of Ozone on Plant Competition and Structural Diversity of Rhizosphere Microbial Communities in Grassland Mesocosms

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S u m m a r y

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The effects of early season ozone stress on plant-plant interactions and on the structural diversity of rhizosphere bacterial communities were investigated in model plant communities (mesocosms) consisting of plant species typical for low-managed permanent grassland. A phytometer-based approach was chosen for testing comparative (intra- and interspecific) competitive abilities of five different perennials (*Anthoxanthum odoratum, Achillea millefolium, Hypericum perforatum, Rumex acetosa, Veronica chamaedrys*), in which each target species competes with the phytometer (*Poa pratensis*). The mesocosms were exposed in triplicate open-top chambers for about 5 weeks in spring to four different levels of ozone. Following ozone exposure, mesocosms were removed from open-top chambers and placed in ground in the open-field where they overwintered until the repeated early-season ozone exposures in the following growing season. Ecophysiological plant traits and growth variables were measured destructively and non-destructively over the course of the season and the structural diversity of different rhizosphere microbial communities was investigated by use of a cultivation-independent technique for genetic profiling of PCR-amplified small-subunit rRNA genes (SSU rDNA).

Results after the first year of this long-term study indicated that the target species differed significantly in their competitive ability against the phytometer and there were clear differences between the plant species in the genetic profiles of their rhizosphere microbial communities. Ozone stress induced symptoms of foliar injury in *Achillea millefolium* and affected the competitive ability *of Veronica chamaedrys* against *Poa pratensis*.

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Introduction

Tropospheric ozone (O_3) is currently regarded as the most prevalent and damaging air pollutant to which vegetation is exposed in many parts of the world (YUNUS & al. 1996). Evidence from experiments over the last 40 years as well as field observations clearly show that current ambient O_3 levels have the potential to suppress crop yields of sensitive species (FUHRER & al. 1997). More recently, evidence is accumulating that O_3 may also have negative effects on plant species of the semi-natural vegetation (DAVISON & BARNES 1998). However, the impacts of O_3 on plant communities, community structure and functioning at the ecosystem level and implications for biodiversity remain poorly understood. The overall ecological effect of O_3 on herbaceous plant communities (especially with perennial species) may not only include above-ground plant growth and productivity, but also root growth and related root processes (BENDER & WEIGEL 2002). An impact on below-ground plant processes may affect soil biological processes e.g. by interacting with soil microbial communities in the rhizosphere and in the bulk soil. To date, however, there is hardly any information available if direct effects of O_3 on plant growth and/or plant community structure are translated into indirect effects on soil biological processes. The present study was therefore performed to investigate the long-term effect of O_3 stress on plant-plant interactions and on the structural diversity of rhizosphere bacterial communities in grassland mesocosms. The mesocosms were exposed to O_3 early in the growing season to test the role of competition as an amplifier of early seasonal stress by O_3 in plant communities.

Material and Methods

A phytometer-based approach was chosen for testing comparative competitive abilities of five different perennials typical for extensively managed permanent grassland (*Anthoxanthum odoratum, Achillea millefolium, Hypericum perforatum, Rumex acetosa, Veronica chamaedrys*), in which each target species competes with a phytometer (*Poa pratensis*), resulting in 11 different types of mesocosms: the five species in monoculture, the five species in mixture with the phytometer, and a monoculture of the phytometer. All plants were grown from seeds and seedlings were selected for uniform development prior to transplantation to the mesocosm-containers that were filled with a natural sandy soil. Each mesocosm consists of four plants, forming either monocultures or the mixed cultures, in which the target species was located in the centre of the mesocosm, and three plants of the phytometer were planted in a triangle as neighbours around the target species. In order to simulate early-season O_3 stress all mesocosms were exposed in open-top chambers for five weeks in May/June 2000 to four different O_3 levels: non-filtered (NF) ambient air, NF plus 25 ppb O_3 (NF+) (8 h day^{-1}; 11:00 - 19:00), NF plus 50 ppb O_3 (NF++), and charcoal-filtered (CF) air plus 25 ppb O_3 (CF+). The latter was considered as a control treatment representing a natural O_3 background concentration. Following O_3 exposure, mesocosms were removed from open-top chambers and were placed in ground in the open-field where they overwintered until the repeated early-season O_3 exposure in the 2001 growing season (4-wks in April/May). The resulting O_3 doses for the 5-wk O_3 exposure in 2000 were 0, 1.68, 6.68, and 11.4 ppm.h (AOT40) and for the 4-wk exposure period in 2001: 0, 0.04, 3.57, and 8.09 ppm.h for CF+, NF, NF+, and NF++, respectively (experiment 1).

An additional set of mesocosms with an identical experimental design as described for experiment 1 was established in 2001 (experiment 2). Mesocosms of experiment 2 were exposed to O_3 for the first time through May/June 2001 (5-wks) The AOT40 values for this exposure period

were 0, 0.44, 4.64, and 9.01 ppm.h in CF+, NF, NF+ and NF++ treatments, respectively. In both experiments, symptoms of foliar injury and leaf senescence were assessed during and after the exposure periods, and plant growth variables of the phytometer and the target species, respectively, were measured destructively and non-destructively over the course of the season.

Samples of the rhizosphere microbial community were taken from the monocultures of experiment 1 after five weeks of O_3 exposure in 2000. At harvest, only the inner core of the established root system was used and soil particles attached to the roots were removed by washing them in water. The microbial cells were extracted with sterile saline solution (0.85 % NaCl), and the cell suspension was centrifuged at 3,600 x g for 30 min. The supernatants were discarded and the pellets were stored at –70°C. The DNA of the collected cell consortia was extracted using the method described by SCHWIEGER & TEBBE 1998. The obtained DNA was purified using a commercially available kit (Wizard DNA clean-up, Cat. #A7280, Promega, Mannheim, Germany) and stored at –20°C. For genetic profiling the V4-V5 region of the eubacterial 16S rRNA genes were PCR amplified using universal primers (Com1; Com2 - 5`-phosphorylated) and SSCP analysis was carried out as described by TEBBE & al. 2001.

Results and Discussion

In a first approach we tested the comparative competitive abilities between species without O_3 stress on the basis of the proportion of the phytometer biomass in mixture with the target species in the control treatments (CF+), i.e. a higher proportion in the phytometer biomass in mixture compared to *Poa pratensis* monocultures indicates a low species' competitive ability against the phytometer, a low biomass indicates a high competitive ability of the species in competition with *Poa pratensis*. Table 1 shows that our target species differed significantly in their competitive ability already in the first experimental year. *Veronica chamaedrys* was found to be the strongest competitor followed by *Achillea millefolium*, *Rumex acetosa* and *Anthoxanthum odoratum*. *Hypericum perforatum* exhibited a very low

Table 1. Mean total above-ground biomass (g dry matter) of the phytometer plants (*Poa pratensis*) in mixed culture with the target species and in monoculture (edge plants) with *Poa pratensis* itself in the control treatments (CF+). Numbers followed by the same letter in each column are not significantly different at $P < 0.05$; d.a.e. = days after emergence.

Competing species	Experiment 1		Experiment 2
	1st year (118 d.a.e)	2nd year (433 d.a.e.)	1st year (127 d.a.e)
V. chamaedrys	10.96 *a*	11.39 *a*	6.44 *c*
P. pratensis	11.09 *a*	13.38 *ab*	7.39 *d*
A. millefolium	11.98 *ab*	15.95 *bc*	4.29 *a*
R. acetosa	13.05 *bc*	17.10 *c*	4.00 *a*
A. odoratum	13.36 *c*	16.63 *c*	5.25 *b*
H. perforatum	14.89 *d*	18.29 *c*	8.76 *e*

competitive ability as indicated by a strong suppression of this species by *Poa pratensis*. These differences in the biomass of the phytometer in mixture with the other species remained in almost the same order after re-growth in the following season. In experiment 2, the phytometer biomass in all mixtures, except for *Hy-*

pericum perforatum, decreased relative to the *Poa* monocultures, indicating high competitve abilities even for *Achillea millefolium*, *Rumex acetosa* and *Anthoxanthum odoratum*. However, experiment 1 and experiment 2 differed significantly in the growing conditions of the phytometer after the establishment of the mesocosms, because of a slower initial growth and reduced tillering and, consequently, lower biomass of *Poa pratensis* in all mesocosms of experiment 2 (Table 1).

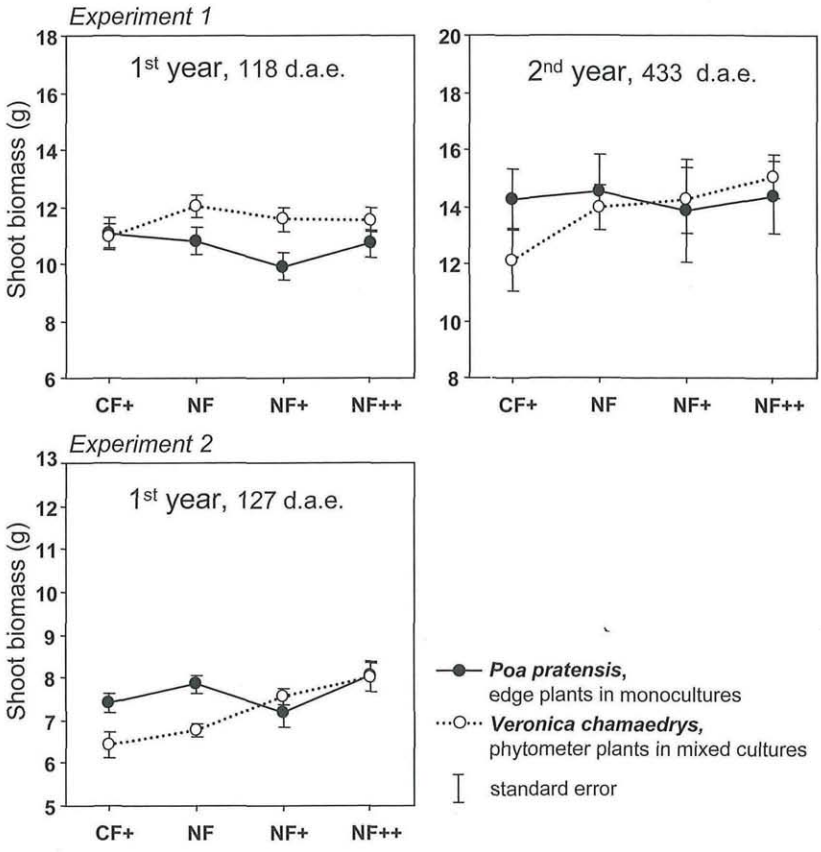

Fig. 1. Effects of different early-season O_3 treatments on above-ground biomass (g dry matter) of the phytometer plants (*Poa pratensis*) in mixed culture with *Veronica chamaedrys* and in monoculture (edge plants) with *Poa pratensis* itself. Plants from experiment 1 were harvested at 118 d.a.e. (days after emergence) and 433 d.a.e, respectively, and from experiment 2 at 127 d.a.e.

In both experiments, early season O_3 stress induced symptoms of foliar injury in *Achillea millefolium*. In experiment 2, *Rumex acetosa* also responded to O_3 stress with symptoms of enhanced senescence of the leaves, which confirms results from other studies in showing that a relatively high sensitivity to O_3 expo-

sures exists within the genera *Rumex* (BERGMANN & al. 1999). There were no O_3 effects on the total above-ground biomass of species grown in monoculture (data not shown). Also, the mixed cultures of experiment 1 did not respond to O_3 during the first growing season. In contrast, O_3 had a significant effect on the competitive response of *Veronica chamaedrys* in competition with the phytometer in the 2001 growing season, and this effect occurred independently in the two separate experiments (Fig. 1). Above-ground biomass of the phytometer in mixed cultures with *Veroncia chamaedrys* from the control treatments (CF+) was significantly lower at 433 days after emergence (d.a.e.) in experiment 1 and at 127 d.a.e. in experiment 2 compared with the phytometer biomass in *Poa* monocultures (without *Veronica chamaedrys*), i.e. that *Veronica chamaedrys* depressed the phytometer growth under non-stressed conditions. However, this competitive advantage of *Veronica chamaedrys* disappeared with increasing O_3 stress (Fig. 1), suggesting a negative impact of O_3 on the competitive ability of *Veronica* against the grass.

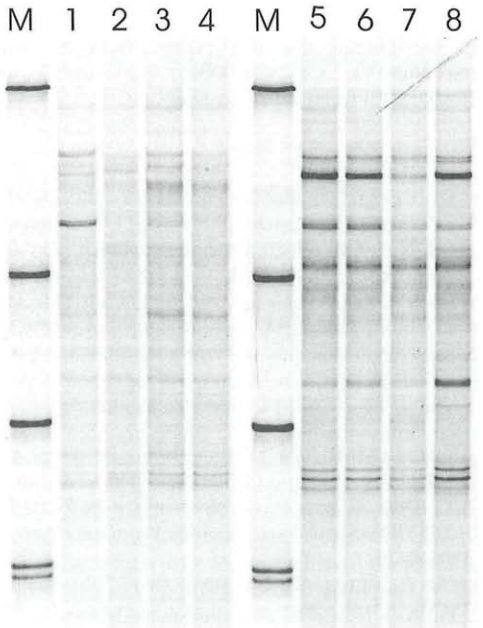

Fig. 2. Silver stained non-denatured polyacrylamide gel showing community profiles of rhizospheres of *Achillea millefolium* (lanes 1 to 4) and *Rumex acetosa* (lanes 5 to 8); four replicates at a time. The coding strands of PCR amplified V4-V5 region of the 16S rRNA genes (~400b) were separated according to their single-strand-conformation-polymorphism. M (= marker): species specific standard of different bacteria. From top: *Bacillus licheniformis*, *Rhizobium trifolii*, *Flavobacterium johnsoniae*, *Rhizobium radiobacter* (formerly *Agrobacterium tumefaciens)*.

Preliminary analyses of the rhizosphere microbial community in the first growing season showed that the SSCP-profiles of the communities in all five plant

species were different (Fig. 2). The genetic profiles showed characteristic patterns of different conformations of DNA fragments. Assuming that there is a correlation between the number of bands and species richness, and between the position of the band and species identity, different microbial communities evolved. This indicated that each plant species created an individual rhizosphere environment selecting for specific microbial communities. Within the genetic profiles of the different communities some DNA-fragments occur non-specifically on several plant species but others appear only on single plant species. Since all plants were grown in the same soil a high potential of bacterial species was present, but only a small part was selected to colonize the rhizosphere of the individual plant species. Early season O_3 stress did not affect the genetic profiles of the communities in the first growing season, however, further analyses will show if there are long-term below-ground O_3 effects.

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