

## **Plant species with different competitive abilities affect microbial growth in rhizosphere**

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### **ABSTRACT**

The aim of this study was to examine how different competitive abilities of two plant species, *Fragaria vesca* and *Duchesnea indica*, alter the functions of rhizosphere microorganisms in dependence on N availability. By intraspecific competition of the plants, significantly lower microbial biomass was observed in the rhizosphere of weak competitive *F. vesca* as compared with strong competitive *D. indica* under N-limiting conditions. However, microbial specific growth rates were 2.4 folds greater in rhizosphere of *F. vesca* than of *D. indica*. By interspecific competition of both plants, microbial growth rates were similar to those for *D. indica* indicating that strong competitive plant species control microbial community in the rhizosphere. The competition of both plant species and the dominating effect of *D. indica* on microbial specific growth rates disappeared after N fertilization. We conclude that competitive ability of *F. vesca* and *D. indica* and their effects on microbial growth rates in rhizosphere are pronounced mainly under N-limitation.

**KEYWORDS:** plant – microbial interactions, growth strategies, N limitation, rhizosphere

### **1. INTRODUCTION**

Rhizosphere - one of the most important 'hot spots' in soil - is characterized by accelerated turnover of microbial biomass and nutrients (Nannipieri, 2003). Composition and functioning of microorganisms in the rhizosphere depend on the quantity and quality of C sources released into the soil by roots (Bolton *et al.*, 1992; Grayston *et al.*, 1998).

As root exudation of plants strongly differ in quantity and composition, some plant species growing in the community might intensively affect rhizosphere microorganisms than the others. Such mediating effect might increase the competitive ability of the plants by structuring the community of rhizosphere microorganisms.

Interspecific competition in the rhizosphere occurs both at population level (plant species-specific interactions, microbial species-specific interactions) and at community level ('plant – microbial' interactions) as well. Interactions in the rhizosphere between plants and microorganisms may be governed by competition for N as it is the most limiting nutrient in natural ecosystems. It was found that grassland plants acquired N more effectively than soil microorganisms (Harrison *et al.*, 2008), but plants and microorganisms might avoid competition for N by utilization of differential N forms, i.e. mineral and organic. So, it is still unclear, how plants with various competitive abilities alter the functions of rhizosphere microorganisms, and competition for N. The aim of this study was to examine how plant species with different strategies growing in intraspecific and interspecific competition affect the functions of rhizosphere microorganisms as dependent on N availability.

## 2. MATERIALS AND METHODS

Two species of strawberry which have similar growth strategy and biology: *Fragaria vesca* L. ('space capturing' species), and *Duchesnea indica* (Andrews) Focke ('space occupation' species) were grown in microcosms with a volume of 310 cm<sup>3</sup> in a temperature controlled greenhouse. Each microcosm was filled with a 50:50 mixture of slightly loamy soil and quartz sand to decrease N availability of the soil. Prior to potting, soil was passed through a 5 mm sieve and watered to field capacity. The chosen pot size ensured that the roots would be able to fill the whole space to get in competition during the 65 days of growth.

A two factorial experiment was established. The first factor was the plant species competition. 15 days old plants of each species were put in microcosms 1) as 4 plants of the same species – intraspecific competition, or 2) as 2 x 2 plants (2 *D. indica* x 2 *F. vesca*) – interspecific competition.

The second factor was N availability. For the 'high N' treatment 20 ml of solution with concentration 42 mM l<sup>-1</sup> KNO<sub>3</sub> and 9 mM l<sup>-1</sup> Ca(NO<sub>3</sub>)<sub>2</sub> were added three times a week. For the "low N" treatment the added amount of N was reduced by the factor of 100. Microcosms were set up to provide three replicates for each competition and each N treatment, yielding a total of 18 microcosms.

At the end of the experiment, the plants were cut, washed, separated in leaves, shoots, roots and stolons, dried at 60 °C in an oven for three days, weighted and ground.

Microbial biomass and the kinetic parameters of microbial growth in the rhizosphere were estimated by dynamics of CO<sub>2</sub> emission from the soil amended with glucose and nutrients (Blagodatsky *et al.*, 2000). Ten grams (dry weight) of soil were amended with a powder-mixture containing glucose (10 mg g<sup>-1</sup>), talcum (20 mg g<sup>-1</sup>), and mineral salts: (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> – 1.9 mg g<sup>-1</sup>, K<sub>2</sub>HPO<sub>4</sub> – 2.25 mg g<sup>-1</sup>, and MgSO<sub>4</sub>·7H<sub>2</sub>O – 3.8 mg g<sup>-1</sup> and the CO<sub>2</sub> production rate was measured hourly at 22 °C using an automated infrared-gas analyzer system.

**Specific growth rate** ( $\mu$ ) of soil microorganisms was estimated by fitting the parameters of the equation:

$$\text{CO}_2(t) = A + B \times \exp(\mu \times t), \quad (1)$$

to the measured CO<sub>2</sub> production rate ( $\text{CO}_2(t)$ ) after glucose addition, where *A* is the initial respiration rate uncoupled from ATP production, *B* the initial rate of the growing fraction of total respiration coupled with ATP generation and cell growth, and *t* time (Blagodatsky *et al.*, 2000).

## 3. RESULTS

### 3.1. Plant species-specific competition

Under N rich conditions *D. indica* had greater total plant biomass compared to *F. vesca* both in intra- and interspecific competition. Under N limitation, however, the total plant biomass of both species was similar. The effects of N on roots were opposite to that on above ground biomass: Greater root biomass of *F. vesca* compared to *D. indica* was observed at N limitation, while high N availability smoothed these differences. Related to the root biomass *D. indica* had a higher specific uptake at low N soil content. *D. indica* was characterized by a higher shoot-to-root-ratio. The N content in all plant organs was only affected by N supply but not by the competition or species. Nitrogen limitation at 'low N' treatment revealed itself by 3.5 times lower N content in plant tissues as compared with 'high N' treatment.

### 3.2. Microbial competition

The patterns of respiratory curves were steeper in the rhizosphere of *F. vesca* versus *D. indica* under N limitation (Fig. 1 A). At high N availability, however, the patterns of respiratory response of rhizosphere microorganisms of both individual plant species were similar (Fig. 1 B). Under N-limiting conditions microbial biomass in the rhizosphere of *F. vesca* was 13% lower than under *D. indica* (Fig. 2 A). By interspecific competition of both plants microbial biomass was also significantly lower as compared with single species of *D. indica*. High level of N smoothed these differences: microbial biomass under plants with intra- or interspecific competition was nearly the same.

At N limitation the specific microbial growth rates were 2.4 folds higher in rhizosphere of *F. vesca* than of *D. indica* (Fig. 2 B). Microbial growth rates for the interspecific competition of both plants were similar to those for *D. indica*. Again, N fertilization eliminated all significant differences in microbial specific growth rates for plants grown in intra- and interspecific competition.

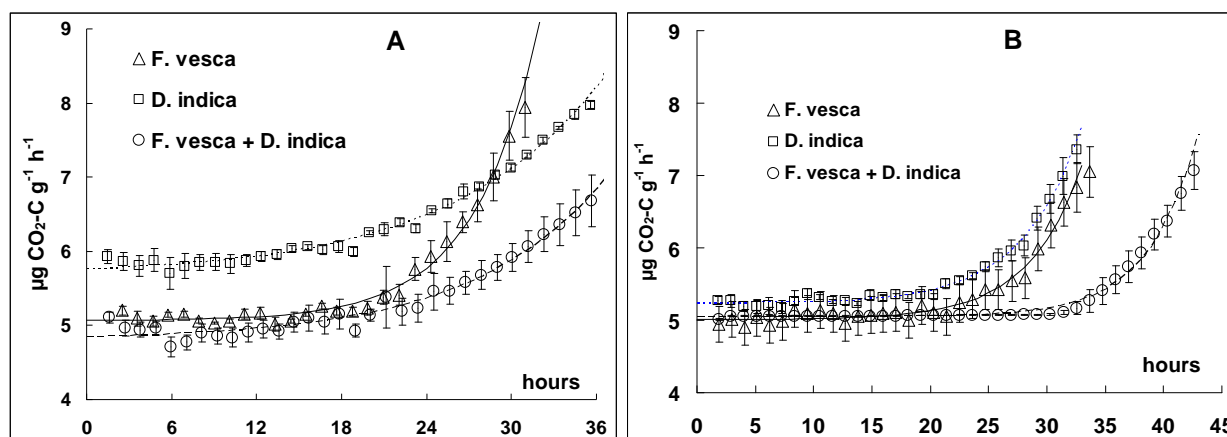


Figure 1. Kinetics of microbial respiration in the rhizosphere of *F. vesca* and *D. indica* growing at low (A) and at high (B) levels of nitrogen. Bars represent standard deviations.

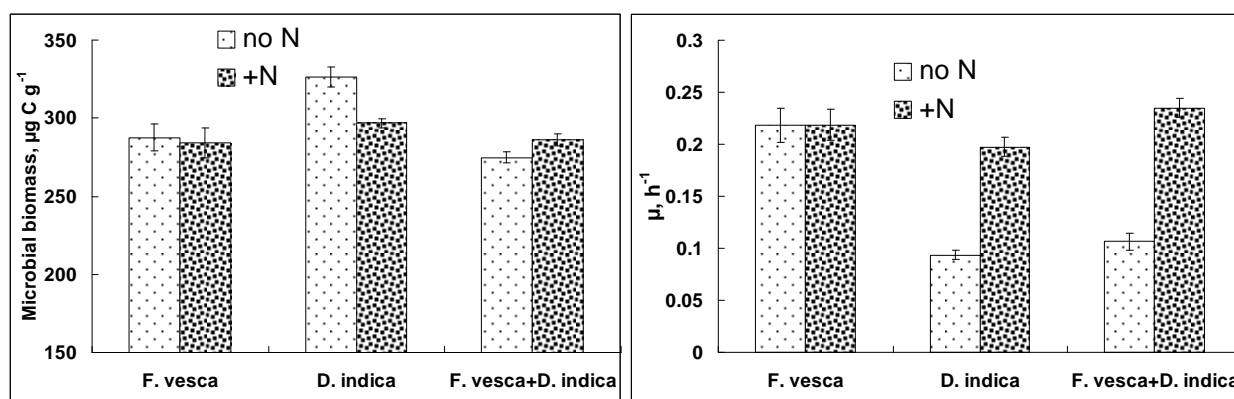


Figure 2. Microbial biomass (A) and specific growth rates (B) of microorganisms in the rhizosphere of *F. vesca* and *D. indica*. Bars represent standard deviations.

#### 4. DISCUSSION

At high N availability the root biomass of both plants was similar while higher total plant biomass of *D. indica* than those of *F. vesca* was observed. We conclude that in the absence of N limitation *D. indica* benefited in the competition with *F. vesca* mainly because of shoot biomass. Such competition, however, did not affect microbial communities in rhizosphere of both plants.

When N was limited the total biomass of *D. indica* decreased sharply while only slight decrease in total biomass of *F. vesca* was observed. The adaptation strategy of *F. vesca* to N limitation revealed itself as an increase in growth of fine roots. Better fine roots proliferation and higher amounts of root exudates resulted in benefiting of fast growing microorganisms with r-strategy and thus, in faster turnover in the rhizosphere of *F. vesca* as compared with *D. indica*. Weaker fine roots development of *D. indica* versus *F. vesca* at low N level caused the specific conditions with lack of available substrate (root exudates) and in nutrients. Dominating of slow growing microorganisms with K-strategy under N limiting conditions caused strong decrease in specific microbial growth rates in the rhizosphere of *D. indica*.

By interspecific competition of both plants at low N level the microbial growth rates were similar to those for *D. indica*. So, growth strategy of microbial community in the rhizosphere of competing plants was mediated mainly by *D. indica* indicating domination in the interspecific competition and stronger effect of *D. indica* on soil microorganisms. Our study revealed the linkage between growth strategies of rhizosphere microorganisms and different adaptation strategies of *F. vesca* and *D. indica* to N limitation.

Since high N level smoothed the differences between plant species in root and microbial biomass as well as in microbial growth rates, we conclude that competitive abilities of plant species studied were responsible for microbial growth in rhizosphere only under N limitation. As it is common that fine root proliferation and root exudation decrease at high N level, N addition smoothed the differences in microbial growth independently on plant competitive abilities.

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