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Plant traits regulating N capture define microbial competition in the rhizosphere

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ABSTRACT

Global warming and nitrogen (N) deposition promote the displacement of native plant species by neophytes which have similar ecological niches but stronger competitive abilities. It remains unclear how plants with different competitive abilities alter microbial growth and turnover in the rhizosphere under high and low N input. We hypothesized 1) slower microbial growth in the rhizosphere of plants with smaller roots and 2) restriction of microbial growth under low versus high N amendment. These hypotheses were tested on two strawberry species: *Fragaria vesca* (native species) and *Duchesnea indica* (an invasive plant in central Europe) grown under intra-specific and inter-specific competition at very low and high N levels.

Species-specific traits of plant–microbial interactions mitigated N deficiency in the rhizosphere. At low N addition the native species *F. vesca* stimulated faster microbial growth and turnover than *D. indica*. *F. vesca* did this by increasing root mass and exudation at the expense of the shoots. In contrast, the invasive plant – *D. indica* – did not increase root mass under low N amendment, but did increase its N uptake rate. This resulted in N deficiency, retarding microbial growth and turnover in the rhizosphere, as revealed by the dominance of slow-growing microorganisms.

A low N level in the soil promoted root growth and rhizodeposition and thus accelerated microbial turnover correspondingly to increasing root mass. Fast N uptake by roots, however, may lead to N deficiency and did retard microbial growth in the rhizosphere. In conclusion, the plant species with the stronger competitive ability at low N level controls the microbial community in the rhizosphere.

1. Introduction

Global warming and nitrogen (N) deposition promote the invasion of neophytes (i.e. plant species non-native to a geographical region) and the displacement of native plant species that have similar ecological niches but lower competitive abilities under new conditions. These plant community changes alter the structure and functioning of the below-ground microbial community, especially in the rhizosphere – one of the most important 'hot spots' in soil. This designation accurately describes this microhabitat: it is characterized not only by an accelerated turnover of microbial biomass and nutrients [34], but also by strong competition both at the population level (plant species-specific, microbial species-specific interactions) and at the community level (plant–microbial interactions). At the community level, plant species and even individual plants determine the composition of the rhizosphere microbial community [21,23]. Remarkably, under inter-specific competition, low-biomass plant neophytes (e.g. grasses) influence the below-ground microbial community even more profoundly than do dominant high-biomass shrub species due to greater N acquisition by low- versus high-biomass plants [37]. As N is a key growth-limiting nutrient in natural ecosystems [45], the competitive strategy of microorganisms depends both on interactions with the plant community and on N availability [20]. At high N availability, grassland plants acquired N less efficiently than soil microorganisms [22]. N limitation increases the amount of exudates released [30], thus affecting the rhizosphere microorganisms'

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depolymerization of N-containing polymers. This increases the fraction of organic N uptake by plants [42]. Depending on the intensity of N limitation, the root growth may decrease [39] or, in contrast, increase (especially that of fine roots [25]. It remains unclear how plants with various competitive abilities alter the functions of rhizosphere microorganisms and competition for N. Despite the wide range of studies examining plant–microbial interactions, a quantitative evaluation of the microbial growth and competitive abilities in the rhizosphere is currently lacking [17,40].

The lack of estimations characterizing the functional parameters of microbial growth kinetics is due to the absence of direct methods for satisfactorily estimating microbial growth *in situ*. An indirect approach suitable for estimating microbial growth parameters is based on the kinetics of substrate-induced growth respiration [10,44]. This approach characterizes the growth rates of a whole microbial community according to the microbial growth model proposed by Ref. [35]. The model reflects the transition of soil microorganisms from a 'sustaining' [43] to an active state, considering both the lag-phase and phase of exponential growth after substrate addition. Although the method requires adding large amounts of substrate to provide exponential (unlimited) microbial growth, *the fraction of microorganisms initially active in un-amended soil* is characterized by the kinetic parameters such as microbial specific growth rate (μ), lag-time and fraction of active microbial biomass. Furthermore, the turnover rate of active microbial biomass calculated by μ (see Methods section) is linked to the intensity of nutrient cycling in microbial community [7]. Thus, the microbial turnover rate indirectly indicates the relative intensity of N uptake and release by microorganisms.

A comparison of the effects on rhizosphere microorganisms is especially meaningful between plants with similar biology and ecological niche requirements but contrasting competitive abilities [8,9]. The Indian mock strawberry [*Duchesnea indica* (Andrews) Focke] is an invasive plant in central Europe. Its spontaneous distribution in Germany, Austria and Switzerland is positively correlated with the average annual temperature [32]. Thus, a warming climate could promote its distribution range. To measure its competitive ability, *D. indica* can be compared with *Fragaria vesca* L., a native species with similar growth strategy and biology. Both are perennial herbs belonging to the Rosaceae family and spread effectively via runners. Based on the differences in root anatomy [1], which may affect nutrient and water transport rates and thus determine the competitive ability for below-ground resources, we selected these species to evaluate their effect on rhizosphere microorganisms. We hypothesized: 1) slower microbial growth rates and turnover in the rhizosphere of plants with smaller root biomass, and 2) a greater effect of N availability on microbial growth in the rhizosphere of plants with high competitive abilities.

The competition belowground is revealed at both the **population level**: competition between (inter-specific) and within (intra-specific) plant species, and at the **community level**: plant–microbial interactions. Both levels of competition become more acute under conditions of nutrient limitation. This study therefore evaluates the effects of plants with different competitive abilities – *F. vesca* L. and *D. indica* growing in intra-specific and inter-specific competition – on changes of belowground microbial growth and turnover depending on level of N amendment.

2. Methods

2.1. Experimental design

Two species of strawberry – *F. vesca* L. and *D. indica* (Andrews) Focke – were grown in microcosms with a volume of 310 cm^3 in a temperature-controlled greenhouse (mean temperature 19 °C). Each microcosm was filled with a 50:50 mixture of soil and quartz sand to decrease the N availability of the soil (slightly loamy stagnic gleysol, C_{total} 0.6%, N_{total} 0.05%, pH 5.1 from grassland at the Ecological- Botanical Garden of the University of Bayreuth). Prior to potting, soil was passed through a 5 mm sieve and watered to 70% of water holding capacity. The chosen microcosm size ensured that the roots fill the whole space and achieve a competitive situation during the 65 days of growth.

A two factorial experiment was established. The first factor was the plant species competition. Fifteen-day-old plants of each species were placed in microcosms 1) as 4 plants of the same species – intra-specific competition, or 2) as 2 × 2 plants (2 *D. indica* × 2 *F. vesca*) – inter-specific competition.

The second factor was N availability. For the 'high N' treatment, 16.8 mg N per microcosm were added as 20 ml of aqueous KNO_3 solution three times a week. N was supplied as nitrate to reduce plant–heterotroph competition for NH_4^+, to shift plants to relying on NO_3^- for their N [42], and to ensure that all N was available and was not adsorbed at the soil matrix. For the "low N" treatment the added amount of N was reduced by a factor of 100. Microcosms were set up to provide three replicates for each competition and each N treatment, yielding a total of 18 microcosms.

When required, microcosms were filled up with water up to 70% water holding capacity. All plants had nearly the same access to light and no aboveground competition existed due to the relatively small size of the plants. The microcosm's location was randomized weekly. Competitive abilities of plants for N were evaluated by N uptake rates using ^{15}N-labelling. For this, 33 days after planting, a stable isotope-labelled ^{15}N nutrient solution containing 2.2% ^{15}N (for high N) or 21.2% ^{15}N (for low N) of the total NO_3^- concentration of the nutrient solution was added to the microcosms (see Ref. [33]; for details). The ^{15}N uptake was calculated by:

$$\frac{\left({}^{15}N_t - {}^{15}N_{control}\right)}{A} \cdot \frac{N_t}{100\ t}$$

where ${}^{15}N_{control} = {}^{15}N/{}^{14}N$ Atom percent of unlabelled plant; ${}^{15}N_t = {}^{15}N/{}^{14}N$ Atom percent 25 days after application of ^{15}N enriched nutrient solution; N_t = N content (mmol gdw^{-1}) 25 days after application of ^{15}N enriched nutrient solution; $t = 25$ days; A – enrichment factor (6.14 and 57.95 for high and low N, respectively).

2.2. Analyses

After 65 days the plants were cut, washed, separated in leaves, shoots, roots and stolons, dried at 60 °C for three days, weighed and ground. The relative abundance of ^{15}N and the total N content in the plant material was analysed using an C–N analyzer (CE Instruments, Milano, Italy) coupled via a ConFlo III to isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany).

Fresh soil samples from each microcosm were used after destructive sampling to estimate microbial biomass and the kinetics of substrate-induced respiration.

Microbial biomass and the parameters of microbial growth kinetics in the rhizosphere were determined based on the dynamics of CO_2 emission from the soil amended with glucose and nutrients (see details in Ref. [15]. In brief, 10 g (dry weight) of soil were amended with a powder-mixture containing glucose (10 mg g^{-1}), talcum (20 mg g^{-1}), and mineral salts: $(NH_4)_2SO_4$ – 1.9 mg g^{-1}, K_2HPO_4 – 2.25 mg g^{-1}, and $MgSO_4 \cdot 7H_2O$ – 3.8 mg g^{-1}. As it was shown in our previous studies the shift in specific growth rates reflecting the ratio between *r*- and *K*-strategists depends on the choice of test substrate and can be better revealed by studying growth on simple than on rich substrate mixtures [5]; 2010). That is why we applied the glucose–mineral mixture, which is commonly