European Journal of Soil Biology 61 (2014) 41–48

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Original article

Plant traits regulating N capture define microbial competition in the rhizosphere

Evgenia Blagodatskaya [a,e,*], Johanna Littschwager [b], Marianna Lauerer [c], Yakov Kuzyakov [a,d]

[a] Dept. of Soil Science of Temperate Ecosystems, University of Göttingen, Germany
[b] Dept. of Agroecosystem Research, BayCEER, University of Bayreuth, Bayreuth, Germany
[c] Ecological-Botanical Gardens, University of Bayreuth, Bayreuth, Germany
[d] Dept. of Agricultural Soil Science, University of Göttingen, Germany
[e] Institute of Physicochemical and Biological Problems in Soil Science, Russian Academy of Sciences, Pushchino, Russia

ARTICLE INFO

Article history:
Received 9 August 2013
Received in revised form
8 December 2013
Accepted 6 January 2014
Available online 19 January 2014
Handling editor: Bryan Griffiths

Keywords:
Plant–microbial interactions
K and *r* growth strategies
N limitation
Rhizosphere microorganisms
Microbial turnover
Rhizodeposition

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'

* Corresponding author. Dept. of Soil Science of Temperate Ecosystems, University of Göttingen, Germany. Tel.: +49 551 3912294; fax: +49 551 39 3310.
E-mail address: janeblag@mail.ru (E. Blagodatskaya).

1164-5563/$ – see front matter © 2014 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejsobi.2014.01.002

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:

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

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

Table 1
Characteristics of *F. vesca* and *D. indica* plants grown for 65 days at low and high N supply under intra- and inter-specific competition. Values are means ± standard errors.

	Intra-specific interactions				Inter-specific interactions			
	F. vesca		*D. indica*		*F. vesca*		*D. indica*	
N Amendment	Low N	High N	Low N	High N	Low N	High N	Low N	High N
Plant biomass, g	0.73^{bc}±0.05	0.79^{bc}±0.05	0.70^{cd} ± 0.04	1.11^{a} ± 0.05	0.71^{cd} ± 0.06	0.93^{ab} ± 0.09	0.60^{c} ± 0.03	1.0^{ab} ± 0.07
Shoot-to-root ratio	1.72^{d} ± 0.11	3.4^{b} ± 0.25	2.16^{cd} ± 0.1	4.72^{a} ± 0.34	1.76^{d} ± 0.12	3.56^{b} ± 0.27	2.33^{c} ± 0.24	6.32^{a} ± 0.77
N uptake rate, μg N g^{-1} roots day^{-1}	0.45^{d} ± 0.03	30.9^{ab} ± 4.3	0.69^{c} ± 0.03	33.3^{ab} ± 2.8	0.47^{d} ± 0.06	27.5^{b} ± 2.9	0.62^{c} ± 0.02	38.8^{a} ± 6.2
N uptake by total root mass, μg N day^{-1}	0.129^{d} ± 0.01	5.94^{b} ± 0.18	0.154^{c} ± 0.01	6.99^{a} ± 0.12	0.126^{d} ± 0.04	5.65^{b} ± 0.13	0.116^{d} ± 0.01	5.63^{b} ± 0.31

used to induce the respiratory activity of soil heterotrophic microorganisms [2]. The glucose–mineral mixture ensures unlimited exponential microbial growth in soil [10,44]) and it is usually applied for estimation of microbial growth parameters. Glucose amounts sufficient for unlimited exponential growth of microorganisms were estimated in preliminary experiments. The amount of mineral salts was selected so that the substrate changed the soil pH by < 0.1 [6]. After addition of the glucose–talcum mixture, the soil samples were immediately placed into plastic 50-mL tubes and the rate of CO_2 production was measured. Each sample was continuously aerated (100 ml min^{-1}) at 22 °C and the evolved CO_2 was measured hourly using an automated infrared-gas analyzer system (24 channels Gas Exchange Measurement System 2250, ADC, UK).

Microbial biomass C (C_{mic}) was calculated according to [2] using the initial substrate-induced respiration (SIR) rate (vCO_2) and the conversion factor of 30 suggested by Ref. [27]:

$$C_{mic}\left(\mu g\ g^{-1}soil\right) = 30 \times vCO_2\ \left(ml\ g^{-1}soil\ h^{-1}\right) \quad (1)$$

Specific growth rate (μ) of soil microorganisms was estimated by fitting the parameters of the equation:

$$CO_2(t) = A + B \times \exp(\mu \times t) \quad (2)$$

to the measured CO_2 production rate ($CO_2(t)$) after glucose addition, where A is the initial respiration rate uncoupled from induced ATP production, B the initial rate of the growing fraction of total respiration coupled with ATP generation and cell growth, and *t* time [10]. The duration of the lag period (t_{lag}) was determined as the time interval between the glucose addition and the moment when the increasing rate of growth-related respiration exceeded the rate of respiration uncoupled from ATP generation by >5%. The estimation of generation time (T_g), i.e. time when biomass was doubled, as well as the estimation of turnover time (T_t) for actively growing microbial biomass consuming glucose was based on specific growth rates [7], i.e:

$$T_g = \ln(2)/\mu;\ T_t = 1/\mu \quad (3)$$

2.3. Statistics

Data were analysed using STATISTICA (version 7.0, StatSoft, Tulsa, OK). One-way ANOVA was performed to test the significance of differences in plant characteristics at intra- and inter-specific competition. The effects of plant community and N level on soil microorganisms were assessed by two-way ANOVAs with plant community composition and N treatment as independent factors. The Tukey test was used to evaluate the significance of treatments at $P < 0.05$. Normality test was applied to confirm normal distribution of the variables.

3. Results

3.1. Competition at a population level: plant species' response to the level of N amendment

At a high level of N amendment, *D. indica* had greater total plant biomass (Table 1) and similar root mass as compared with *F. vesca* (Fig. 1). At low N, both species did not differ significantly in total mass, whereas the root mass was significantly higher for *F. vesca.* Thus, both plants decreased shoot mass at low versus high N treatment (Fig. 1). However, *F. vesca* increased root mass to overcome N limitation, while the root biomass of *D. indica* was not changed significantly at low versus high N level. The shoot-to-root ratio for both species was 2–3 times greater at high versus low N (Table 1). This, however, had different reasons in the two species: the decrease in shoot-to-root ratio at low N versus high N (Table 1) reflected the increase in root mass in *F. vesca* and the decrease in shoot mass of *D. indica* (Fig. 1).

Higher shoot mass at high N level (Fig. 1) caused a higher shoot-to-root ratio for *D. indica* versus *F. vesca* both in intra- and inter-specific competition (Table 1) indicating more efficient growth of aboveground biomass of *D. indica*. With one exception (high N, intra-specific competition), *D. indica* was characterized by significantly faster N uptake rate (estimated by ^{15}N uptake) per g of root mass than *F. vesca* (Table 1). Despite *F. vesca* increased the root mass at low N level, the N uptake rate estimated for total root mass in intra-specific interactions was still higher for *D. indica* (Table 1). The contribution of N to the roots (as a percentage of total N content) was greater for *F. vesca* at both levels of N availability in intra-specific competition. Intra- or inter-specific competition did not affect the N distribution in the plants (data not shown).

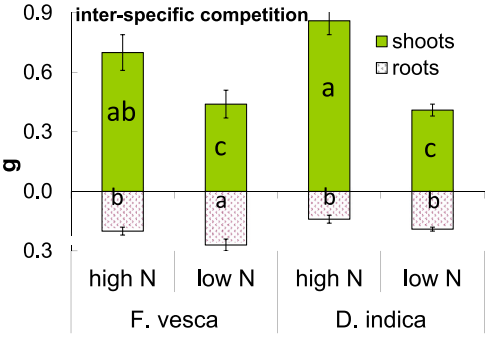

Fig. 1. Effect of N level on shoot and root mass of *F. vesca* and *D. indica* grown under inter-specific competition. Bars represent standard errors of means. Different letters show significant effects ($P < 0.05$).

3.2. Competition at a community level: effect of plants on rhizosphere microorganisms

Total microbial biomass

In intra-specific competition and under N-limiting conditions, the total microbial biomass was 13% lower in the rhizosphere of *F. vesca* versus *D. indica* (Fig. 2, top). At low N level the microbial biomass was significantly lower in inter-specific plant competition compared with single species of either *D. indica* or *F. vesca.* High N level, however, smoothed these differences: microbial biomass under plants with intra- and inter-specific competition was nearly the same. A significant effect of N on microbial biomass was observed for *D. indica* in intra-specific competition. Unexpectedly, microbial biomass in the rhizosphere of *D. indica* was even higher at low than at high N level (Fig. 2, top).

Microbial respiration and specific growth rates

The initial respiratory response to addition of glucose and nutrients was higher under N limitation versus unlimited N conditions only for microorganisms in the rhizosphere of *D. indica* (Fig. 3, top). No significant differences in initial respiration after glucose addition were observed between high and low N treatments in the rhizosphere of *F. vesca* and at inter-specific competition of both plants (Fig. 3, middle, bottom).

An exponential increase of respiration indicating unlimited microbial growth due to the added glucose was observed in soils from all treatments after a lag period (Fig. 3). The patterns of respiratory curves were steeper in high versus low N treatments in the rhizosphere of *D. indica* and at inter-specific competition of both plants (Fig. 3, top, bottom). This indicates faster microbial growth at the absence of N limitation. Indeed, the *specific microbial growth rates* calculated according to Eq. (2) were twice greater at high N than at low N for *D. indica* and for inter-specific plant competition (Fig. 2, bottom).

Under N limitation the μ-values were 2.4-fold higher in the rhizosphere of *F. vesca* than of *D. indica* grown individually

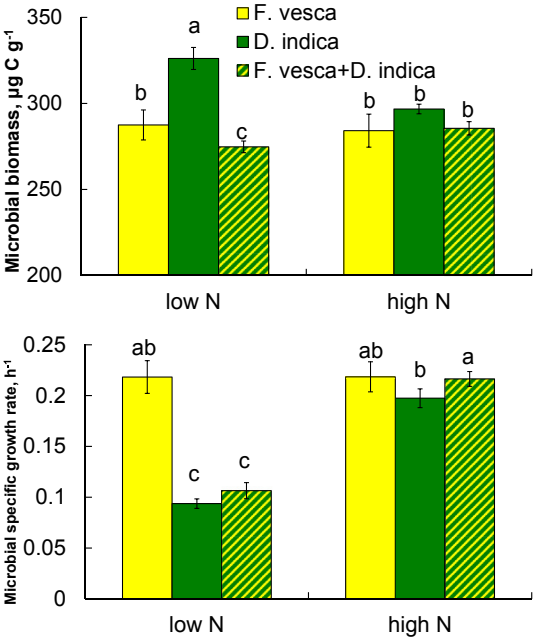

Fig. 2. Microbial biomass determined by substrate-induced respiration (top) and microbial specific growth rates (bottom) in the rhizosphere of *F. vesca* and *D. indica* grown at low and high N supply levels under intra- and inter-specific competition. Bars represent standard deviations of means ($n = 3$). Different letters show significant effects ($P < 0.05$).

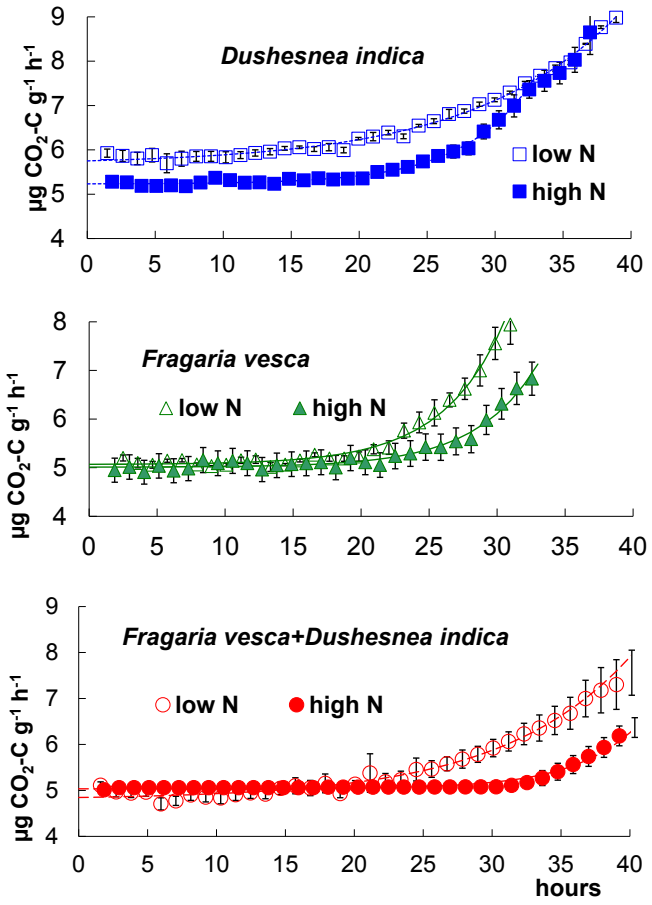

Fig. 3. Kinetics of microbial respiration in the rhizosphere of *D. indica* (top) and *F. vesca* (middle) as well as their inter-specific competition (bottom) at low (low N) and at high (high N) levels of nitrogen. Bars represent standard deviations of means ($n = 3$). Symbols represent measured values; lines are fitted according to Eq. (2).

(Fig. 2, bottom). Microbial growth rates in the inter-specific competition situation were similar to those of *D. indica* (Fig. 2, bottom), indicating the domination of *D. indica*. Thus, in inter-specific competition at low N level microbial growth was mediated by *D. indica.*

High N availability had no effect on microbial growth rates in the rhizosphere of *F. vesca* (Fig. 2, bottom), but strongly increased the specific growth rate of rhizosphere microorganisms of *D. indica.* Again, N fertilization eliminated all significant differences in microbial specific growth rates for plants grown in intra- and inter-specific competition (Fig. 2, bottom).

Lag period, microbial generation and turnover time

The shortest lag period (the delay between glucose addition and exponential growth, Table 2), slowest microbial growth rate, but highest microbial biomass (Fig. 2) were observed for the rhizosphere of *D. indica* at low N. This indicated that the microbial biomass amount rather than specific growth rates determined the duration of the lag period. N addition prolonged the lag period for microorganisms in the rhizosphere of both plant species. Maximal values of T_{lag} occurred at the high N level in inter-specific plant competition (Table 2).

Faster microbial growth and turnover in the rhizosphere of *F. vesca* grown singly at low N was revealed by 3–4 h shorter generation time (T_g) and by 5–6 h shorter microbial turnover time (T_t) as compared with the rhizosphere of *D. indica* and with the rhizosphere of both plant species growing together (Table 2). At a

Table 2
Characteristics of microbial growth in the rhizosphere of *F. vesca* and *D. indica* grown for 65 days at low and high N supply under intra- and inter-specific competition. Values are means ± standard errors.

	Intra-specific interactions				Inter-specific interactions	
	F. vesca		*D. indica*		*F. vesca* and *D. indica*	
N amendment	Low N	High N	Low N	High N	Low N	High N
Lag time, h	$19.8^{d} \pm 0.6$	$23.6^{bc} \pm 0.4$	$15.6^{e} \pm 0.8$	$22.5^{c} \pm 0.3$	$20.1^{d} \pm 0.1$	$32.5^{a} \pm 0.6$
Generation time, h	$3.18^{b} \pm 0.63$	$3.17^{b} \pm 0.58$	$7.40^{a} \pm 0.99$	$3.51^{b} \pm 0.44$	$6.51^{a} \pm 1.31$	$3.20^{b} \pm 0.29$
Turnover time, h	$4.6^{b} \pm 0.3$	$4.6^{b} \pm 0.3$	$10.7^{a} \pm 0.5$	$5.1^{b} \pm 0.2$	$9.4^{a} \pm 0.7$	$4.6^{b} \pm 0.16$

high N level, both the generation time and the turnover time did not differ significantly between the species.

According to the two-way ANOVA, the effect of both factors – plant community composition and N application rate, as well as their interactions – were responsible for 77.3% of the variation in specific growth rates (μ) and for 67.6% in the microbial biomass amount (Table 3). The effect of N on μ values was even greater than the effect of plant species (Table 3). In contrast, the effect of N on total microbial biomass C was insignificant (Table 3), whereas the plant community was mostly responsible for microbial biomass variation.

3.3. Effect of plant adaptation strategy to N level on microbial growth

Our study revealed a different adaptation strategy of *D. indica* and *F. vesca* to the N level. At high N availability the root biomass of both plants was similar (Fig. 1), while *D. indica* had a higher total plant biomass (Table 1). We conclude that without N limitation *D. indica* benefited compared with *F. vesca* allocating more N to shoots [33]. Such benefit of *D. indica* at high N availability however, did not affect biomass (Fig. 2, top), and microbial growth parameters (Table 2) in the rhizosphere of both species. When N was limited the shoot biomass of *D. indica* decreased sharply, whereas root mass did not change significantly in comparison with the high N level. In contrast, N limitation did not alter total biomass of *F. vesca* (Table 1), but increased the root mass by 1.5 times at low versus high N level (Fig. 1). As a result, low N availability reduced microbial growth rate in the rhizosphere of *D. indica*, whereas microbial growth in the rhizosphere of *F. vesca* was not altered by N deficit (Fig. 2, bottom).

4. Discussion

4.1. Root-mediated functioning of microbial communities under competition for N

We showed that *D. indica* had a smaller root biomass, a lower N content in roots, a faster N uptake, and a greater shoot-to-root ratio compared to *F. vesca*. Accordingly, at the population level (competition between plant species), *D. indica* is stronger competitor for N than *F. vesca*, especially in N-rich environments [33]. At the community level (plant–microbial competition), however, the high competitive abilities of *D. indica* for N affected the soil microorganisms only at N limitation. This indicated different species-specific mechanisms of plant–microbial interactions under N-limited conditions.

To determine whether the functioning of rhizosphere microorganisms was mediated by roots, we related the root mass and N uptake rate by plants to the microbial turnover time (Fig. 4). At low N amendment, the turnover of rhizosphere microorganisms increased with the increasing root mass (Fig. 4, top). This supports the evidence that plants increase their fine root development and root exudation under higher competition for N [31,41,46]. The increased supply of root exudates alters competitive interactions between different microbial functional groups [19] and favours those microorganisms with faster turnover and shorter generation times [7]; 2010). The generation time T_g for microbial biomass growing on glucose (Table 2) was only several hours longer than that calculated for continuous pure cultures at optimal temperatures (1.5–2 h [11], and than that in the rhizosphere of *Zea mays* L. (2–3 h [7]. This can be explained by the different growth period of the studied plants: 2 weeks for *Z. mays*, >2 months for strawberry plants. This indirectly reveals the linkage between the stage of root system development and the turnover of rhizosphere microorganisms [47].

Plant–microbial competition for N is mediated not only by root mass but also by the N uptake rate. The microbial turnover decreased with increasing N uptake rate by plants (Fig. 4, bottom) because of higher N limitation. This demonstrates the potential for plants themselves to modify microbial competition for N at the species level [22]: they select for specific microbial communities by altering the quantity and quality of resources entering the soil [4,48].

4.2. r and K-selection – refuted theory or suitable tool?

The adaptation strategy of *F. vesca* to N limitation was manifested as increased root growth; this means it allocates more C belowground than does *D. indica*. Better root proliferation and more root exudates benefited fast-growing microorganisms with higher μ values which, according to acknowledged ecological concepts, are usually classified as *r*-strategists [3,6].

Due to the specifics of soil heterogeneity, direct application of *r*- and *K*-selection theory at the level of microbial communities is restricted by the difficulties in quantitatively determining *K*-values, i.e. the carrying capacity of soil microhabitats, especially if the microbial mortality rate is unknown. Nonetheless, this empirical concept is well accepted in soil biochemistry and has a wide area of application at the level of population/community dynamics [16,36]. Thus, we refer to the *r*-/*K*-selection concept because it helps explain the mechanisms of plant–microbial competition in our study. Accelerated microbial turnover (corresponding to shorter generation times) in the rhizosphere of *F. vesca* versus *D. indica* at low N supported the domination of the fast-growing *r*-species in the rhizosphere of the former.

Table 3
Contribution (%) of plant community composition (plant), and of N application rate (N), to variation of microbial specific growth rates (μ) and total microbial biomass estimated by two-way ANOVA for rhizosphere microbial communities of *F. vesca* and *D. indica* grown at intra- and inter-specific competition.

Source of variation	Specific growth rate	Total microbial biomass
Plant	32.0*	63.4*
N	45.3*	4.2
Unexplained	22.8	32.4

*Contribution significant at $P < 0.05$.

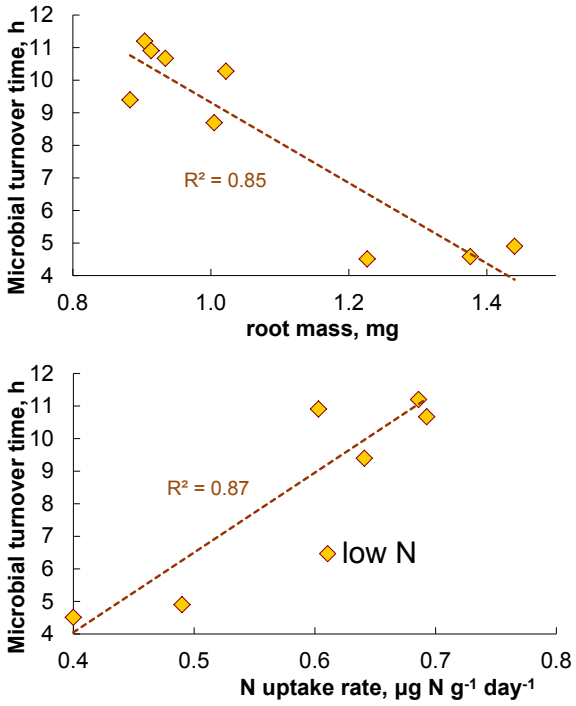

Fig. 4. Relationship between the root dry mass (top) and N uptake rates by plants (bottom) and microbial turnover time in the rhizosphere of *F. vesca* and *D. indica* in the low N treatment.

Weaker root development of *D. indica* versus *F. vesca* at low N level corresponds to a smaller amount of available root exudates, benefiting those microorganisms with a *K*-strategy. *D. indica* increased N limitation in the rhizosphere at low N and thereby, caused a shift to domination of slow-growing microorganisms with *K*-strategy. Thus, instead of an expected acceleration of microbial growth rates, which is common in the rhizosphere, our study revealed the intriguing situation that microbial growth rates were retarded in the rhizosphere of a competitive plant at low N availability. Such a situation illustrates how competition between plants at the population level can affect plant–microbial competition at the community level: as a long-term effect of N limitation, highly competitive plants may cause a shift in microbial strategy to slow-growing microorganisms which, despite their relatively long generation times, maintain high population densities under limiting conditions [36]. This is precisely what our study revealed: the largest microbial biomass and longest generation time were observed in the rhizosphere of *D. indica* at low N level, illustrating that *K*-strategists have an advantage over *r*-strategists when the population density is close to the carrying capacity of the environment (here by N). Hence, plants growing under N limiting conditions benefited the fraction of *K*-strategists in soil microbial communities. For rhizosphere microorganisms, the transition from species with *r*- to *K*-growth strategy serves as an adaptation mechanism and more efficient metabolism at N limitation. Thus, similarly to observations by Ref. [19]; our study showed that different plant adaptation strategies to N limitation are manifested in opposite responses of root mass production and exudation, thus differently affecting microbial communities in the rhizosphere [14].

Our previous studies revealed an increase in the contribution of *r*-strategists in the rhizosphere [7]; 2010). Here, we underline the importance of differentiating the two situations: i) comparison of rhizosphere and root-free soil, and ii) N and C limitation. The microbial turnover and fraction of *r*-strategists increase in the rhizosphere compared to root-free soil [8,9,13]. We therefore took the next step and compared the growth strategies in the rhizosphere of two plants (with no root-free soil comparison). Importantly, the well-known surplus of available C in the rhizosphere benefits microbial *r*-strategists only at a sufficient N level.

Our study demonstrated the applicability of the general ecological theory of the *r*- to *K*-selection continuum [38] to quantitatively assessing plant-mediated microbial competition in the rhizosphere. This competition resulted in the relative domination of fast- or slow-growing species depending on plant traits regulating N capture.

4.3. Possible consequences of plant invasion for soil C stock

Under conditions of inter-specific competition at low N level, the microbial growth rates were similar to those for *D. indica* alone. Accordingly, strong microbial competition for N in the rhizosphere of the 'space occupation' plant *D. indica* caused the selection of slow-growing *K*-species (due to low amounts of available N and C); their enzymes systems are able to degrade low-available organic substrates and acquire N [12]. Thus, domination of *K*-strategists can accelerate the decomposition of soil organic matter, i.e. the priming effect [18], resulting in long-term soil C loss. Similarly, in an N-limited grassland, root-mediated proliferation of Gram(+) bacteria and a decline in the fungal and Gram(−) species was concurrent to an accelerated mineralization of soil organic matter [26]. Such changes in community structure indicate the transition in microbial growth strategy we observed in our study. Therefore, enhanced evolution of CO_2 from soil organic matter and a reduced organic carbon supply to the soil by roots can be the indirect consequences of spontaneous invasion of 'space occupation' plant species. This has potential feedbacks with climate change.

4.4. Effect of N amendment on plant–microbial competition

At high N level, *r*-strategists benefited independently of plant competitive abilities (Fig. 2 bottom). When the N source is not limited, microorganisms compete more effectively than plant roots for organic and inorganic N in soil over the short term [22,24]. This benefits fast-growing microorganisms with a low use efficiency [40]. Note, however, that N addition smoothed the impact of the limiting factor and smoothed the differences between plant species in root mass [33] and in microbial biomass; it also reduced the differences in the microbial growth rates in the rhizosphere of both plant species. The absence of a relationship between either root mass or N uptake rate and microbial turnover at a high N level can be due to less fine root proliferation and less root exudation [28,29]. We conclude that the studied plant species affected microbial growth and turnover only under N limitation.

5. Conclusions

Our study revealed that strategies of plant species to mitigate N deficit by root–microbial interactions depend on plant traits regulating N capture. Plants can actively control microbial metabolism by changing root mass, rhizodeposition and/or N uptake rate. This results in plant-specific rhizosphere microbial communities [23].

The root mass of the native species (*F. vesca*) increased under N limitation to compensate for the lack of nutrients, maintaining the fast-growing microorganisms (Fig. 5). Faster microbial turnover in the rhizosphere of *F. vesca* boosts the N release from microbial cells, thus increasing available N and the N uptake by roots. Accordingly, under N limitation, plants with low competitive abilities at the community level stimulate the rhizosphere microorganisms to an

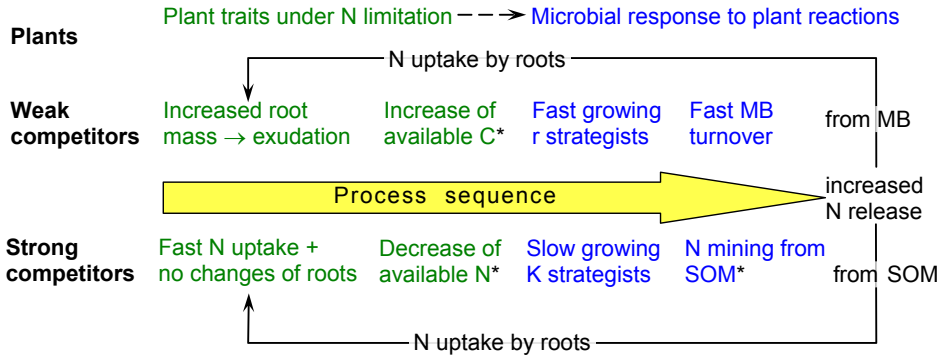

Fig. 5. Conceptual scheme on plant–microbial interactions at low N level in soil depending on plant competitiveness for N acquisition. Weak competitors release more available C by roots to stimulate microorganisms for fast turnover and for release of N from microbial biomass. In contrast, fast N uptake by strong competitors forces microorganisms to mineralise N from SOM. In both ways plants mitigate N limitation in the rhizosphere, but the additional N is released from microbial biomass turnover by weak competitors and from SOM by strong competitors. The processed labelled with * are conceptual – they were not measured in this study.

accelerated microbial turnover; this releases N back into the rhizosphere.

In contrast, the invasive species – *D. indica* – did not change root mass under N limitation, but was highly competitive for N uptake. This caused stronger N limitation and more strongly reduced available C compared to the rhizosphere of *F. vesca*. This, in turn, altered the structure of the rhizosphere microbial community, specifically by benefiting slow-growing microorganisms with a *K*-strategy involving higher N and C use efficiency (Fig. 5). The switch from an *r*- to *K* microbial growth strategy mediated by this plant – a strong competitor for N – points to a species-specific mechanism of plant–microbial interactions. The result is a more efficient utilization of limited N by rhizosphere microorganisms.

The invasive plant species with its strong competitive ability for N changed the below-ground microbial community to a domination of slow-growing, *K*-selected species, they can degrade low-available organics and thus have access to additional N sources. Domination of *K*-strategists can accelerate the decomposition of soil organic matter, i.e. promote the priming effect (Fig. 5). Thus, the invasion of 'space occupying' plant species has two impacts: it can reduce the C input into the soil compared to native species, and it can potentially cause positive feedback with climate change by accelerating the decomposition of soil organic matter and releasing additional CO_2.

Note that the observed differences in specific growth rates are merely indirect evidence for a shift within the *r*- and *K*-continuum of soil microbial communities. Our investigation thus provides interesting perspectives for future studies, which should combine kinetic respiration analysis and RNA-based estimations of microbial community structure.

Acknowledgements

The study was supported by European Commission (Marie Curie IIF program, project MICROSOM), by DAAD (German Academic Exchange Service) and by Russian Academy of Sciences (Leading Scientific School Program-No 6123.2014.4).

References

[1] P. Albornoz, M. Arias, A. Castagnaro, J.C. Díaz Ricci, Comparative root anatomy of *Duchesnea indica*, *Fragaria vesca* and *Potentilla tucumanensis* (Rosaceae) in Tucumán province, Argentina, Adansonia 29 (2007) 255–267.
[2] J.P.E. Anderson, K.H. Domsch, A physiological method for the quantitative measurement of microbial biomass in soils, Soil Biol. Biochem. 20 (1978) 107–114.
[3] J.H. Andrews, R.F. Harris, r and K-selection and microbial ecology, in: K.C. Marshall (Ed.), Advances in Microbial Ecology, vol. 9Plenum Press, New York, 1986, pp. 99–144.
[4] R.D. Bardgett, T. Streeter, R. Bol, Soil microbes compete effectively with plants for organic-N inputs to temperate grasslands, Ecology 84 (2003) 1277–1287.
[5] E.V. Blagodatskaya, O.S. Khokhlova, T.-H. Anderson, S.A. Blagodatskii, Extractable microbial DNA pool and microbial activity in Paleosols of Southern Urals, Microbiology 72 (2003) 750–755.
[6] E.V. Blagodatskaya, S.A. Blagodatsky, T.-H. Anderson, Y. Kuzyakov, Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies, Appl. Soil Ecol. 37 (2007) 95–105.
[7] E.V. Blagodatskaya, S.A. Blagodatsky, T.-H. Anderson, Y. Kuzyakov, Contrasting effects of glucose, living roots and maize straw on microbial growth kinetics and substrate availability in soil, Eur. J. Soil Sci. 60 (2009) 186–197.
[8] E. Blagodatskaya, S. Blagodatsky, M. Dorodnikov, Y. Kuzyakov, Elevated atmospheric CO_2 increases microbial growth rates in soil: results of three CO_2 enrichment experiments, Global Change Biol. 16 (2010a) 836–848.
[9] E. Blagodatskaya, J. Littschwager, M. Lauerer, Y. Kuzyakov, Growth rates of rhizosphere microorganisms depend on competitive abilities of plants and N supply, Plant Biosyst. 144 (2010b) 408–413.
[10] S.A. Blagodatsky, O. Heinemeyer, J. Richter, Estimating the active and total soil microbial biomass by kinetic respiration analysis, Biol. Fertil. Soil 32 (2000) 73–81.
[11] S.A. Blagodatsky, M. Kesik, H. Papen, K. Butterbach-Bahl, Production of NO and N_2O by the heterotrophic nitrifier *Alcaligenes faecalis parafaecalis* under varying conditions of oxygen saturation, Geomicrobiol. J. 23 (2006) 165–176.
[12] R. Chen, E. Blagodatskaya, M. Senbayram, S. Blagodatsky, O. Myachina, K. Dittert, Y. Kuzyakov, Decomposition of biogas residues in soil and their effects on microbial growth kinetics and enzyme activities, Biomass Bioenergy 45 (2012) 221–229.
[13] W. Cheng, Rhizosphere priming effect: its functional relationships with microbial turnover, evapotranspiration, and C-N budgets, Soil Biol. Biochem. 41 (2009) 1795–1801.
[14] W.R. Cookson, M. Osman, P. Marschner, D.A. Abaye, I. Clark, D.V. Murphy, E.A. Stockdale, C.A. Watson, Controls on soil nitrogen cycling and microbial community composition across land use and incubation temperature, Soil Biol. Biochem. 39 (2007) 744–756.
[15] M. Dorodnikov, E. Blagodatskaya, S. Blagodatsky, A. Fangmeier, Y. Kuzyakov, Stimulation of r- vs. K-selected microorganisms by elevated atmospheric CO_2 depends on soil aggregate size, FEMS Microbiol. Ecol. 69 (2009) 43–52.
[16] N. Fierer, M.A. Bradford, R.B. Jakson, Toward an ecological classification of soil bacteria, Ecology 88 (6) (2007) 1354–1364.
[17] N. Fierer, A.S. Grandy, J. Six, E.A. Paul, Searching for unifying principles in soil ecology, Soil Biol. Biochem. 41 (2009) 2249–2256.
[18] S. Fontaine, A. Mariotti, L. Abbadie, The priming effect of organic matter: a question of microbial competition? Soil Biol. Biochem. 35 (2003) 837–843.
[19] D.A. Fornara, D. Tilman, S.E. Hobbie, Linkages between plant functional composition, fine root processes and potential soil N mineralization rates, J. Ecol. 97 (2009) 48–56.
[20] C. Freeman, S.Y. Kim, S.H. Lee, H. Kang, Effects of elevated atmospheric CO_2 concentrations on soil microorganisms, J. Microbiol. 42 (2004) 267–277.
[21] U. Hamer, F. Makeschin, Rhizosphere soil microbial community structure and microbial activity in set-aside and intensively managed arable land, Plant Soil 316 (2009) 57–69.
[22] K.A. Harrison, R. Bol, R.D. Bardgett, Do plant species with different growth strategies vary in their ability to compete with soil microbes for chemical forms of nitrogen? Soil Biol. Biochem. 40 (2008) 228–237.
[23] A. Hartmann, M. Schmid, D. van Tuinen, G. Berg, Plant-driven selection of microbes, Plant Soil 321 (2009) 235–257.
[24] A. Hodge, J. Stewart, D. Robinson, B.S. Griffiths, A.H. Fitter, Competition between roots and soil micro-organisms for nutrients from nitrogen-rich patches of varying complexity, J. Ecol. 88 (1) (2000) 150–164.
[25] R. Hyvonen, G.I. Agren, S. Linder, The likely impact of elevated CO_2, nitrogen deposition, increased temperature and management on carbon sequestration

in temperate and boreal forest ecosystems: a literature review, New Phytol. 173 (2007) 463–480.
[26] K. Klumpp, S. Fontaine, E. Attard, X. Le Roux, G. Gleixner, J.-F. Soussana, Grazing triggers soil carbon loss by altering plant roots and their control on soil microbial community, J. Ecol. 97 (2009) 876–885.
[27] E.A. Kaiser, T. Mueller, R.G. Joergensen, H. Insam, O. Heinemeyer, Evaluation of methods to estimate the soil microbial biomass and the relationship with soil texture and organic matter, Soil Biol. Biochem. 24 (1992) 675–683.
[28] Y. Kuzyakov, G. Domanski, Carbon input by plants into the soil. Review, J. Plant Nutr. Soil Sci. 163 (2000) 421–431.
[29] Y. Kuzyakov, H. Ehrensberger, K. Stahr, Carbon partitioning and below-ground translocation by *Lolium perenne*, Soil Biol. Biochem. 33 (2001) 61–74.
[30] Y. Kuzyakov, S.V. Siniakina, J. Rühlmann, G. Domanski, K. Stahr, Effect of nitrogen fertilisation on below-ground carbon allocation in lettuce, J. Sci. Food Agric. 82 (2002) 1432–1441.
[31] G. Lemaire, P. Millard, An ecophysiological approach to modelling resource fluxes in competing plants, J. Exp. Bot. 50 (1999) 15–28.
[32] A. Liefländer, M. Lauerer, Spontanvorkommen von *Duchesnea indica*: Ein Neophyt breitet sich in den letzten Jahren verstärkt aus, Berliner Bayer Botanischer Geselschaft 77 (2007) 187–200.
[33] J. Littschwager, M. Laurer, E. Blagodatskaya, Y. Kuzyakov, Nitrogen uptake and utilization as a competition factor between invasive *Duchesnea indica* and native *Fragaria vesca*, Plant Soil 331 (2010) 105–114.
[34] P. Nannipieri, J. Ascher, M.T. Ceccherini, L. Loretta, P. Giacomo, G. Renella, Microbial diversity and soil functions, Eur. J. Soil Sci. 54 (2003) 655–670.
[35] N.S. Panikov, Microbial Growth Kinetics, Chapman & Hall, London, 1995.
[36] N. Panikov, Microbial ecology, in: L.K. Wang, et al. (Eds.), Handbook of Environmental Engineering, Environmental Biotechnology, vol. 10, Springer Science + Business Media, LLC, 2010, pp. 121–191, http://dx.doi.org/10.1007/978-1-60327-140-0_4.
[37] D.A. Peltzer, P.J. Bellingham, H. Kurokawa, L.R. Walker, D.A. Wardle, G.W. Yeates, Punching above their weight: low-biomass non-native plant species alter soil properties during primary succession, Oikos 118 (2009) 1001–1014.
[38] E.R. Pianka, On r- and K-selection, Am. Nat. 104 (1970) 592–597.
[39] K.S. Pregitzer, D.R. Zak, J. Maziasz, J. DeForest, P.S. Curtis, J. Lussenhop, Fine root growth, mortality, and morphology in a factorial elevated atmospheric CO_2 soil N availability experiment, Ecol. Appl. 10 (2000) 18–33.
[40] J.I. Prosser, B.J.M. Bohannan, T.P. Curtis, R.J. Ellis, M.K. Firestone, R.P. Freckleton, J.L. Green, L.E. Green, K. Killham, J.J. Lennon, A.M. Osborn, M. Solan, C.J. van der Gast, J.P.W. Young, The role of ecological theory in microbial ecology, Nature 5 (2007) 384–392.
[41] X. Raynaud, B. Jaillard, P.W. Leadley, Plants may alter competition by modifying nutrient bioavailability in rhizosphere: a modeling approach, Am. Nat. 171 (2008) 44–58.
[42] J.P. Schimel, J. Bennett, Nitrogen mineralization: challenges of a changing paradigm, Ecology 85 (2004) 591–602.
[43] J.L. Smith, B.L. Mcneal, H.H. Cheng, G.S. Campbell, Calculations of microbial maintenance rates and net mineralization in soil at steady-state, Soil Sci. Soc. Am. J. 50 (1986) 332–338.
[44] J. Stenström, K. Svensson, M. Johansson, Reversible transition between active and dormant microbial states in soil, FEMS Microbiol. Ecol. 36 (2001) 93–104.
[45] P.M. Vitousek, R.W. Howarth, Nitrogen limitation on land and in the sea – how can it Occur, Biogeochemistry 13 (2) (1991) 87–115.
[46] X. Xu, F. Stange, A. Richter, W. Wanek, Y. Kuzyakov, Light affects competition for inorganic and organic nitrogen between maize seedlings and soil microorganisms, Plant Soil 304 (2008) 59–72.
[47] X. Xu, H. Ouyang, A. Richter, W. Wanek, G. Cao, Y. Kuzyakov, Spatio-temporal variations determine plant-microbe competition for inorganic nitrogen in an alpine meadow, J. Ecol. 99 (2011).
[48] D.R. Zak, W.E. Holmes, D.C. White, A.D. Peacock, D. Tilman, Plant diversity, soil microbial communities, and ecosystem function: are there any links? Ecology 84 (8) (2003) 2042–2050.