

Contrasting root behaviour in two grass species: a test of functionality in dynamic heterogeneous conditions

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Abstract Root systems are highly plastic as they express a range of responses to acquire patchily distributed nutrients. However, the ecological significance of placing roots selectively in nutrient hotspots is still unclear. Here, we investigate under what conditions selective root placement may be a significant functional trait that determines belowground competitive ability. We studied two grasses differing in root foraging behaviour, *Festuca rubra* and *Anthoxanthum odoratum*. The plants were grown in stable and more dynamic

heterogeneous environments, by switching nutrient patches halfway through the experiment. *A. odoratum* was a factor of two less selective in placing its roots into nutrient-rich patches than *F. rubra*. *A. odoratum* produced overall higher root length densities with higher specific root length than *F. rubra* and acquired more nutrients. *A. odoratum* appeared to be the superior competitor, irrespective of the nutrient dynamics. Our results suggest that root behaviour consisting of producing high root length densities at relatively low biomass investments can be a more effective foraging strategy than placing roots selectively in nutrient hotspots. When understanding the functionality of root traits among different species, specific root length may play a key role.

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Introduction

Plants respond to nutrients in a heterogeneous environment, by selectively placing roots into nutrient-rich soil patches (Hutchings and de Kroon 1994; Hodge 2004, 2006; Schenk 2006). Selective root placement (also termed root foraging ‘precision’) is defined as the investment in roots placed into nutrient-rich zones, relative to root abundance in nutrient-poor zones. Selective root placement is ubiquitous among terrestrial

species, but the benefits of this trait appear largely context-dependent, as species identity, nutritional composition of patches and duration of study influence the outcome (Hutchings and John 2004; Lamb et al. 2004; de Kroon and Mommer 2006; Kembel et al. 2008). This context-dependency exists for individually-grown plant species (Kembel and Cahill 2005), and also for plants under interspecific competition, where a high degree of selective root placement sometimes (Hodge et al. 1999; Robinson et al. 1999), but not always, leads to the highest competitive ability (Bliss et al. 2002; Cahill and Casper 1999; Fransen and de Kroon 2001; Rajaniemi 2007).

A unifying ecological principle explaining these inconsistencies may be that it is not selective root placement per se that is conferring competitive advantage, but the root length density that is achieved (Robinson 1996; Hodge et al. 1999). Experimental evidence shows that the competitively superior species displayed the highest degree of selective root placement, but also achieved the highest root length density in the nutrient patch (Hodge et al. 1999). Being selective may thus be one way to realize high root length densities, but any other cost-effective phenotypic behaviour with the same result would be equally functional. However, we have insufficient understanding of which alternative combinations of root traits may lead to high root length densities in nutrient hotspots, and under what conditions they are profitable.

A high degree of selective root placement is generally expected to be particularly beneficial for growth in a soil with large, stable patches (Einsmann et al. 1999; Wijesinghe et al. 2005). If large predictable patches are combined with small and unstable patches, the benefits of selective root placement are likely to decrease since time is too short for roots to pre-empt the nutrient patch before it disappears (Fransen et al. 1999). Under such dynamic conditions, we expect that an overall high root length density will be more important for nutrient uptake than a high selectivity in root placement, and a less selective species may then take up a large share of the belowground resources.

The current experimental study was designed to discriminate between the importance of being selective versus achieving high root length densities in general, and to shed light on the underlying plant traits responsible for realizing these densities. The

relative importance of selectivity and different root length densities was manipulated by applying nutrient patches of different size and stability. We investigated intra- and interspecific competition in two common grass species, well known to differ in root foraging behaviour. *Festuca rubra* displays a high degree of selective root placement in response to nutrient-rich patches, whereas *Anthoxanthum odoratum* spreads its roots more evenly in the soil, even if patches with nutrients are present (Fransen et al. 1998; 1999; Fransen et al. 2001). Competitive ability (sensu de Wit 1960) was determined by comparing above-ground biomass of these species in mixtures and monocultures. By comparing root growth of these two species in nutrient-rich and -poor patches, we quantified selective root placement. Moreover, using the ^{15}N tracer technique, we investigated if nutrient uptake from a specific patch (nutrient-rich or -poor) was proportional to the actual root distribution in these species.

We specifically hypothesised:

- 1) A high degree of selective root placement will only increase competitive ability of a species if this results in more root length in the nutrient-rich patch than the competing species. As *F. rubra* has shown to be more selective than *A. odoratum*, *F. rubra* is expected to reach higher root length densities in nutrient-rich patches than *A. odoratum*, particularly when these patches are large and stable.
- 2) Nutrient uptake from a patch is proportional to root length density. As we expect *F. rubra* to place more roots in the nutrient patches, this species will take up more nutrients from the nutrient-rich patches than *A. odoratum*. When the location of part of the patches is changed, *A. odoratum* will take up relatively more nutrients from newly emerging nutrient patches than *F. rubra*, because *A. odoratum* achieves higher root length densities outside the nutrient-rich patches than *F. rubra*. Under such conditions of increased patch dynamics, *F. rubra* will increase root growth in the stable patches.
- 3) Due to stronger selective root placement and the resulting higher nutrient uptake, *F. rubra* will have a competitive advantage over *A. odoratum* in stable patch conditions; this dominance of *F. rubra* will decrease in favour of *A.*

odoratum when (part of) the nutrients are applied in small and unstable patches.

Materials and methods

Species selection and plant material

To investigate the growth consequences of contrasting root behaviours in dynamic heterogeneous conditions, we build upon the knowledge available on root foraging traits in two co-occurring perennial grassland species of European hay meadows: *Festuca rubra* L. and *Anthoxanthum odoratum* L. *F. rubra* is a more precise forager in terms of root placement than *A. odoratum*, reflected in values of selective root placement of 3.5 and 2.4 (roots in rich/roots in homogeneous soil), respectively (Fransen et al 1999). *A. odoratum* is thought to express a higher physiological plasticity than *F. rubra*, measured as ^{15}N uptake per unit root biomass per day, being 2.2 and 1.4 $\text{mg g}^{-1} \text{day}^{-1}$, respectively (Fransen et al. 1998, 1999, 2001). Often, *F. rubra* achieves up to two times higher root densities than *A. odoratum* (Fransen et al. 2001; Mommer et al. 2010), but not always (e.g. Fransen et al. 1998). The species have comparable relative growth rates (Grime and Hunt 1975; Fransen et al. 1998).

Seeds of the two grass species were collected in the river forelands of the river Rhine, near Nijmegen (The Netherlands) and germinated for 10 days on moistened filter paper in Petri dishes. The seedlings were transplanted to small pots (diameter 5 cm) filled with a vermiculite:sand (1:1) mixture and grown for 4 weeks. The seedlings were watered with $\frac{1}{4}$ Hoagland solution three times a week.

Competition treatments and density series

After 4 weeks, the plants were transplanted to the actual experimental units, being bigger pots (diameter 23 cm, depth 16 cm) filled with the same vermiculite:sand (1:1) mixture. Within a pot, four plants were planted in a square, 9 cm apart.

The nutrient distribution treatments (stable, switch; see explanation below) were applied to monocultures and mixtures of the two grass species. Competitive ability was measured by comparing shoot growth in mixture to shoot growth in monoculture (de Wit 1960;

Fransen et al. 2001). Every combination of nutrient treatment (stable or switch) \times competition treatment (mono of *A. odoratum* or *F. rubra* or their mix) was replicated 18–22 times, resulting into 115 pots with plants to be harvested at the end of the experiment. Halfway through the experiment, at the moment of the switch in nutrient supply (see below), 60 pots were harvested.

To investigate whether competition occurred in our four plants per pot setup, we included a density series with two, four and eight plants of both species; the number of replicates was eight. If the plants in our four plants per pot actually competed strongly, total biomass per pot would be similar for four and eight plants per pot (Fransen et al. 2001). The density series only received the so-called stable treatment. Significant competition occurred in the experiment where a density of four plants was used, since final yield (maximum shoot biomass 10.6 and 8.8 g per pot for *A. odoratum* and *F. rubra*, respectively) in a density series had already levelled off between two and four plants per pot.

Nutrient heterogeneity treatment

We explicitly aimed for an experimental setup with multiple nutrient patches that were predictable to different degrees. At first, all plants were grown in pots with a heterogeneous nutrient application for 5 weeks. Thereafter, in half the pots, the nutrient-rich patches were continued as before (referred to as the stable treatment). In the other half of the pots, the nutrient status of some of the patches changed position (referred to as the switch treatment), thus confronting the plants with root investments at the ‘wrong’ location. Plants were then grown for another 4 weeks.

The heterogeneous treatment consisted of three types of patches that were created at the start of the experiment (Fig. 1). First, in the centre of the pot, a stable nutrient-rich patch was created. This nutrient patch, containing half of the nutrients, was permanently available. Second, two nutrient-rich patches were created at the outer side of the pot (i.e. peripheral patches on the horizontal axis in Fig. 1). These patches continued to be nutrient-rich in the stable treatment, but in the switch treatment they changed into nutrient-poor patches after 5 weeks. Third, two nutrient-poor sites were also created at the outer side of the pot (i.e. peripheral patches on the

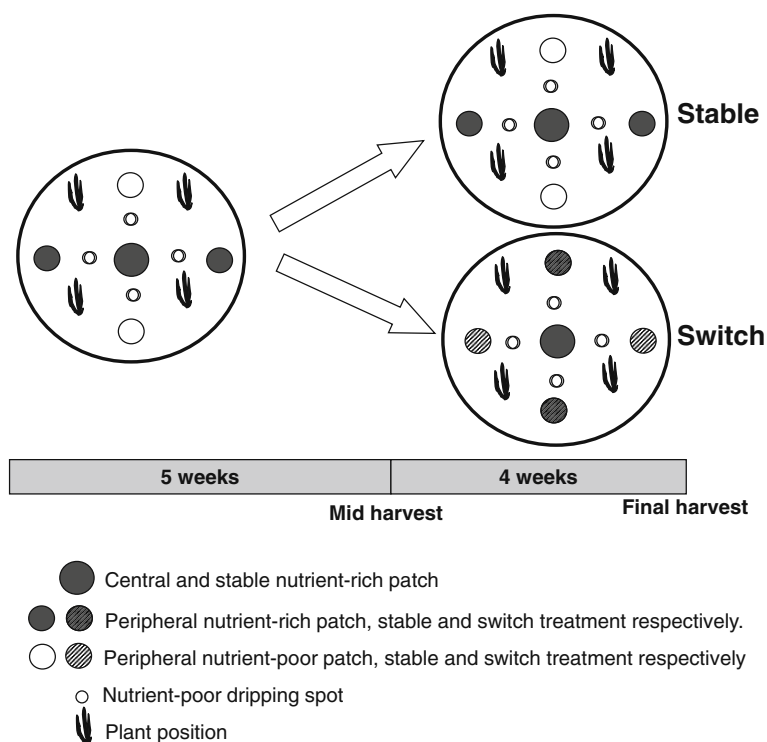


Fig. 1 Schematic representation of the experimental design. The *largest circles* represent the experimental units (pots) containing four grass plants, forming monocultures or 1:1 mixtures. *Smaller circles* represent different nutrient patches, created by adding nutrient solution. The nutrient patch at the centre of the pot (*black*) is continuously nutrient-rich. The peripheral nutrient patches are either nutrient-rich (15 ml 1/2 Hoagland's solution three times a week; *black or dark grey*

vertical axis in Fig. 1). These patches continued to be nutrient-poor in the stable treatment (i.e. background soil nutrient status), but in the switch treatment they changed into nutrient-rich patches after 5 weeks. The combination between switch treatment and position resulted in four different peripheral patch types (rich-rich, poor-poor, rich-poor, poor-rich). The nutrient-rich patch in the centre of the pot received an equal amount of nutrient solution as the two nutrient-rich peripheral patches together, i.e. the stable centre patch was twice as large as each of the peripheral patches. The distance of each plant to the three nearest patches was the same, so the chance that the plants encountered one of the three patches was the same. The switch treatments differed in patch distribution, but received the same total amount of nutrients at any time.

The patches were created using a dripping system with nutrient solution three times a week (cf. Campbell

circles) or nutrient-poor (15 ml 1/64 Hoagland's solution three times a week; *white or light grey circles*). In half the pots, the peripheral patches shifted in nutrient status halfway the duration of the experiment (*open vs. hatched circles*). To prevent leaching of nutrients from one nutrient hotspot into another, poor (15 ml 1/64 Hoagland's solution) was dripped in between the patches at four positions in the pot (*smallest white circles*). For more details see "Materials and methods"

and Grime 1989; Jansen et al. 2006; Visser et al. 2008). The nutrient-rich patches received 15 ml 1/2 strength Hoagland's solution [3 mM KNO_3 , 2 mM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 1 mM $\text{NH}_4\text{H}_2\text{PO}_4$, 0.5 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, Fe-EDTA and micronutrients] and the nutrient-poor patches received 15 ml of a very poor background nutrient solution [1/64 strength Hoagland's, which is lower in nitrate concentration than the Dutch standards for tap water; 0.09 mM KNO_3 , 0.06 mM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 0.03 mM $\text{NH}_4\text{H}_2\text{PO}_4$, 0.015 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, Fe-EDTA and micronutrients]. To prevent leaching of nutrients from one nutrient hotspot in another nutrient patch, nutrient-poor background solution (1/64 strength Hoagland's) was dripped in between the different nutrient-rich patches (cf. Jansen et al. 2006; see Fig. 1).

To quantify the nutrient concentration of the patches, soil nitrate concentration was analysed at different spots in separate pots with and without

plants. Soil cores (5–10 g, diameter 1 cm, 0–10 cm deep) were taken 2 h and 2 days after the nutrient solution had been applied. The soil sample was diluted in 100 ml demineralised water and shaken for 1 h by 120g to dissolve the nitrate in the water. The mixture was filtered and stored in plastic pots in a freezer. The samples were later analysed colourimetrically with a continuous flow analyser for nitrate (Traacs 800+; Brann and Luebbe, Germany). Nutrient-rich patches contained $0.870 \pm 0.089 \mu\text{mol NO}_3^- \text{g}^{-1}$ soil, nutrient-poor patches $0.230 \pm 0.025 \mu\text{mol NO}_3^- \text{g}^{-1}$ soil 2 h after dripping. Nutrient-rich patches contained thus more than 3.5 times more nitrate than nutrient-poor patches, indicating that the dripping method with the nutrient solution in syringes resulted in a heterogeneous soil. In pots where plants were present, all available nutrients were taken up within 2 days (when nitrate concentrations had dropped to $0.031 \pm 0.010 \mu\text{mol NO}_3^- \text{g}^{-1}$ soil) with no significant differences anymore between the patch types. The term ‘dynamic’ used with regard to the switch treatment refers to the complete switch in peripheral position of the nutrient solution halfway through the experiment. However, one may argue that even the permanent patches showed some degree of nutrient dynamics as the nutrients were consumed within 2 days and then reappeared again.

In order to avoid water deficit, all pots were watered once a week with 300 ml of the very nutrient-poor nutrition solution (1/64 Hoagland’s). This background solution was applied 2 h before nutrient addition. The applied nutrient levels were limiting since N contents of *F. rubra* and *A. odoratum* shoots (living and dead biomass) at final harvest were, averaged over all treatments, only 1.3 and 1.1% of dry biomass, which is well below standard values of 2–5% N of well-fed plants (Marschner 1995).

The position of the pots in the greenhouse was arranged randomly to homogenise growth conditions among the replicated plants. The experiment was performed in the heated greenhouse of the Radboud University Nijmegen (20°C, PPFD was between 200 and $550 \mu\text{mol m}^{-2} \text{s}^{-1}$, 16 h/8 h) from February to April 2006.

In summary, we performed a competition experiment with two grass species, growing in monoculture and mixture. All nutrients were applied heterogeneously in three different types of patches in two different ways: stable nutrient heterogeneity or partly dynamic. In order to determine nutrient uptake from

the different patch types, we applied ^{15}N pulses in all the patch types separately. Replicates for ^{15}N application in the three patch types were 6, but root responses could be determined from all pots, leading to a replicate number of 18 (+3–4 for ^{15}N background concentrations).

Measurements

All destructive measurements were performed 5 weeks (mid harvest) and 9 weeks (final harvest) after the treatment had started. Initial plant biomass was also determined at the start of the experiment.

Shoot biomass, root biomass and root length

At mid- and final harvest, shoots were clipped at the root base, dried at 70°C for 48 h and weighed to determine biomass. At the same time, from the same pots, soil cores of 16 cm depth were taken with a device that allowed coring at five locations in the pot simultaneously. The five locations were the centre patch (diameter=56 mm), the two peripheral nutrient-rich patches (diameter=40 mm) and the two peripheral nutrient-poor patches (diameter=40 mm). Note that the position of nutrient-rich versus poor patches in the pot depended on the treatment (see Fig. 1). The two pairs of peripheral patches (with similar nutrient regime) were summed, resulting in three different root sections: one from the nutrient-rich patch in the centre, one from the peripheral rich patches, and one from the peripheral poor patches, all with the same soil volume. The roots in the cores were carefully washed from the soil. It was impossible to visually disentangle the roots from the mixtures by species.

Sub-samples of the roots from the different patches were taken to determine specific root length. These root sub-samples were conserved in 0.01% HgCl_2 solution at 4°C for a few weeks. Before scanning (at 600 dpi; Epson expression 10000 XL; Regent Instruments, Canada), the roots were coloured with a Neutral Red solution (0.5 g L^{-1}) to improve contrast. Scans were analysed with Winrhizo™ software (Regent Instruments, Canada). Afterwards, the scanned root samples were dried at 70°C for 48 h in order to calculate specific root length (m g^{-1}). Selective root placement of the two species and the mixture was expressed as root length density

(m dm^{-3}) in the target patch divided by the root length density of the continuously poor nutrient patch. Root length density was calculated from the root biomass from a patch, specific root length and the soil volume of the respective patch. The remaining roots in the pot were also washed in order to determine total root mass.

^{15}N and total N uptake

We investigated nutrient uptake in the different patch types at both mid and final harvest by labelling with ^{15}N either to (1) the central nutrient-rich patch, (2) the two peripheral nutrient-rich patches or (3) the two peripheral nutrient-poor patches. The three ^{15}N applications were divided over the total of 18 replicates, i.e. 6 replicates per position of ^{15}N application. Three to four replicates were used to determine natural background concentrations of ^{15}N . We added ^{15}N to the nutrient solution in each of the two syringes per patch type, during the last three dripping sessions (i.e. 5, 3 and 1 days) before the final harvest.

For each ^{15}N application, 1 ml demineralised water containing 1 mg ^{15}N (supplied as K^{15}NO_3 ; = 9 μmol ~nitrate equivalent of 15 ml of 1/32 Hoagland's solution) was added to the 'normal' gift of 15 ml of 1/2 Hoagland's solution. In the case of the nutrient poor syringes, ^{14}N -nitrate from the 1/64 nutrient solution was replaced for a ^{15}N equivalent of 1/32 Hoagland's solution (9 μmol ^{15}N in 16 ml demineralised water). Thus, while differences in total nutrient concentration of the patches remained, every patch received an equal amount of ^{15}N . Total amount of K^{15}NO_3 supplies was 6 mg, leading to a total amount of 0.84 mg ^{15}N given per pot, which means that on average 0.21 mg ^{15}N was available per plant (planting density=4). On average, 50% of the supplied ^{15}N to each pot was taken up by the shoots.

For the analysis of both ^{15}N and total N, all shoot material of single plants was dried and pulverized and 2–2,5 mg of this shoot material was used in a nitrogen analyzer (EA 1110, Carlo Erba; Thermo Electron, Milan, Italy) in combination with a mass spectrometer (DeltaPlus; Thermo Finnigan, Bremen, Germany). The natural available background of ^{15}N concentration, determined on every species \times treatment combination (average background ^{15}N concentrations for all species \times treatment combinations 0.367%; no signifi-

cant differences between species and treatments) was subtracted from the ^{15}N concentrations of the labelled plants in order to determine excess ^{15}N concentrations.

Data analysis

The effects of intra- versus inter-specific competition and heterogeneous nutrient dynamics on shoot biomass and total N accumulation were assessed using univariate ANOVA (SPSS 15.0; GLM), with species (*A. odoratum* vs. *F. rubra*), planting (monoculture vs. mixture) and treatment (stable vs. switching dripping regime) as fixed factors. These analyses were performed on species averages per pot ($n=18\text{--}21$).

In statistical analyses using root length density the factors 'species' and 'planting' were combined in one fixed factor (referred to as 'species combination') with three levels: *A. odoratum*, *F. rubra* and 'mixture' (i.e. the response of *A. odoratum* and *F. rubra* as a whole), since the roots of the two species in mixture could not be disentangled quantitatively.

Analyses regarding root length density and ^{15}N uptake included, next to effects of species and planting, respectively, effects of patch type. Since we were not interested in effects of patch size per se, statistical analyses for root length density and ^{15}N uptake were run separately for the centre patches and peripheral patches, although root and nutrient uptake responses to centre and peripheral patches will not have been completely independent. For the centre patches in both stable and switch treatment, nutrient status was always nutrient-rich, since the switching only affected the peripheral patches. For the peripheral patches, the switch treatment and the position of the patches resulted into 4 different patch types with a different nutrient status in time (rich–rich, poor–poor, rich–poor and poor–rich), of which two were within a pot and not completely independent either. In the centre patches, the nutrient status was thus not affected by the switch treatment, whereas the nutrient status was affected by the switch treatment in the peripheral patches. This was an additional reason to run separate statistical analyses for the centre patches and peripheral patches. In order to test the effect of interdependence in the peripheral patch types, we also performed a statistical analysis with patch position nested within treatment (results not shown). As this

yielded basically similar results as the factor patch type with four levels, we chose to present the most simple type of analysis, i.e. four levels of peripheral patch types.

For the monocultures of *A. odoratum* and *F. rubra*, analyses of ^{15}N uptake were performed with root length density as a covariate factor. This covariate factor was not significant as root length density was driven by the factor patch type. Ln transformations of the data were performed when needed to meet the assumptions of ANOVA.

Fig. 2 Selective root placement (-; **a-c**), root length densities (m dm^{-3} ; **d-f**) and specific root length (m g^{-1}) of *A. odoratum* and *F. rubra* monocultures and their mixture in the different patch types in the stable and switch treatment at final harvest. Selectivity is expressed as root investment in the target patch divided by the root investment of the peripheral stable nutrient-poor patch (i.e. estimate for root length density in the background soil). A value of 1 thus indicates no selectivity; the larger the deviation from 1, the higher the degree of selectivity of root placement. Selective root placement is significantly higher in *F. rubra* than in *A. odoratum*. However, this is not due to higher root length density in the nutrient-rich centre or rich-rich peripheral patch, but to a lower root length in poor→poor patches. Specific root length is significantly higher in *A. odoratum* ($n=16$) than in *F. rubra* ($n=18$). Values are means \pm SE per pot

Results

Root distribution in stable and switch nutrient patches

F. rubra was twice as selective in placing its roots in the nutrient rich patches than *A. odoratum* (Fig. 2a,c). However, in contrast to our hypothesis, the larger selectivity of *F. rubra* was not due to higher root length densities in the nutrient hotspots than *A. odoratum* as both species had similar root length densities in the stable nutrient-rich patches (central and peripheral;

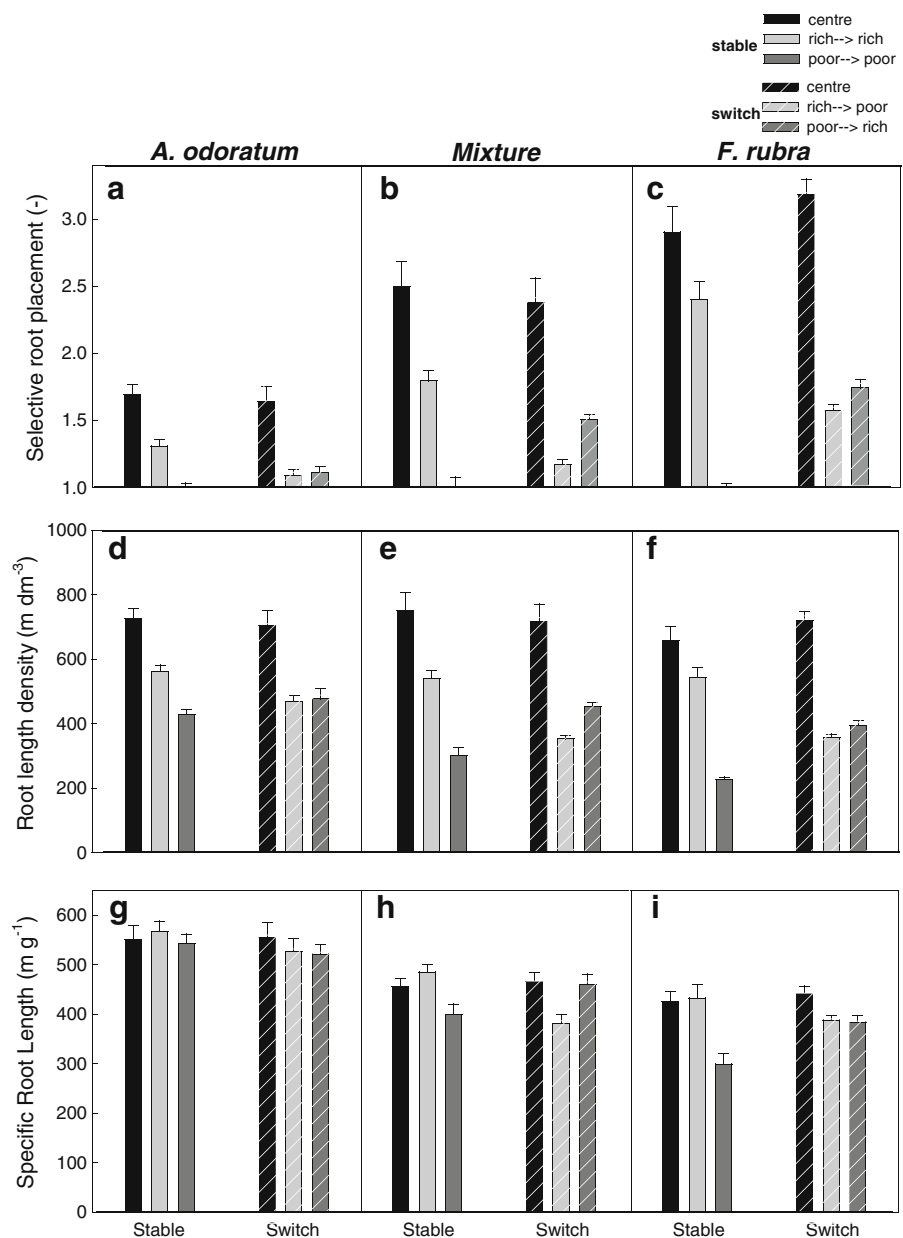


Fig. 2d, f). However, at the end of the experiment, in the stable nutrient-poor patch and the switched peripheral patches, the root length density of *F. rubra* was lower than that of *A. odoratum* (Fig. 2d, f). *A. odoratum* placed its roots more evenly in the background soil than *F. rubra*, as differences in root length density between permanent rich and the poor patch were smaller in the former species.

In general, the total root length of *A. odoratum* was higher than that of *F. rubra*. However, the picture is different when expressed on a per unit of mass basis. Root biomass was significantly lower for *A. odoratum* than for *F. rubra* monocultures. Total root dry mass (patches added to roots in rest of the pot) for these two species was 1.75 ± 0.04 and 1.92 ± 0.03 g, respectively (mean \pm SE; significant species effect; $F_{1,73} = 11.3$, $p < 0.001$). Switching nutrient patches did not affect total root investment (treatment effect $F_{1,73} = 0.1$, $p > 0.05$). The difference in root investments based on length vs. biomass was due to differences in specific root length between the species (Fig. 2g-i). Averaged over all patch types, the specific root length of *A. odoratum* was 30% higher compared to *F. rubra*, i.e. 550 ± 29 and 424 ± 21 m g⁻¹, respectively (mean \pm SE; significant species effect; $F_{1,96} = 137.5$, $p < 0.001$). *A. odoratum* showed no responses to shifts in nutrient application with regard to specific root length, but *F. rubra* did (Fig. 2g-i; Table 1). Specific root length of *F. rubra* was 40% lower in permanent poor patches than in rich patches.

Root length densities in the permanent nutrient-rich centre patch were almost 30% higher than in the smaller permanent nutrient-rich peripheral patches. Root growth in the nutrient-rich centre patch was not affected by the switch treatment of the peripheral patches

(Fig. 2d-f; Table 1). Root length densities in the peripheral patches were affected by patch type (significant effect patch type; Fig. 2d-f; Table 1), indicating that the application of nutrients and the shift therein led to significant differences in root biomass distribution. Root length density in the peripheral patches was highest in the stable nutrient-rich patches, and lowest in the stable nutrient-poor patches. Roots in new nutrient-rich patches did not reach similar densities as in permanent nutrient-rich peripheral patches, but the root length densities in these poor-rich patches were always second highest of the peripheral patches. Root length densities in nutrient-rich patches that became nutrient-poor still increased in the second half of the experiment (Fig. S1), but less so than roots in patches that remained nutrient-rich (Fig. 2d-f). The nutrient shift thus resulted in differences in root length densities in the different nutrient patch types.

Root growth and thus selectivity in the patches of mixture pots were very similar to the averages of the two species monocultures (Fig. 2b, e). There were no indications that interspecific plant-plant interactions affected root density or morphology.

Nutrient uptake in nutrient patches

In monocultures, *A. odoratum* captured significantly more ¹⁵N from the centre patches and the peripheral patches than *F. rubra* (Fig. 3a-d; Table 2). ¹⁵N uptake rates per unit length were similar between the species in the peripheral patches, but significantly different in the centre patches (Fig. 4a; Table 3). There was a non-significant trend towards increased nutrient uptake rates in the poor-rich patches (Fig. 3e, f).

Table 1 ANOVA results (*F* values) of root length density and specific root length in the different patch types at final harvest, analysed separately for the centre patch and the peripheral patches of *A. odoratum*, *F. rubra* and their mixture

Source	Root length density				Specific root length			
	Centre patch		Peripheral patches		Centre patch		Peripheral patches	
	<i>df</i>	<i>F</i> value	<i>df</i>	<i>F</i> value	<i>df</i>	<i>F</i> value	<i>df</i>	<i>F</i> value
Species combination	2	0.53 ^{ns}	2	30.39 ^{***}	2	15.63 ^{***}	2	68.69 ^{***}
Patch type	1	0.00 ^{ns}	3	75.56 ^{***}	1	0.32 ^{ns}	3	9.17 ^{***}
Species*Patch type	2	0.75 ^{ns}	6	5.16 ^{***}	1	0.04 ^{ns}	6	2.79 [*]
Error (MS)	109	38,022	218	6,884	48	4,493	96	3,614

n = 18–21

P* < 0.05, **P* < 0.001, *ns* non-significant

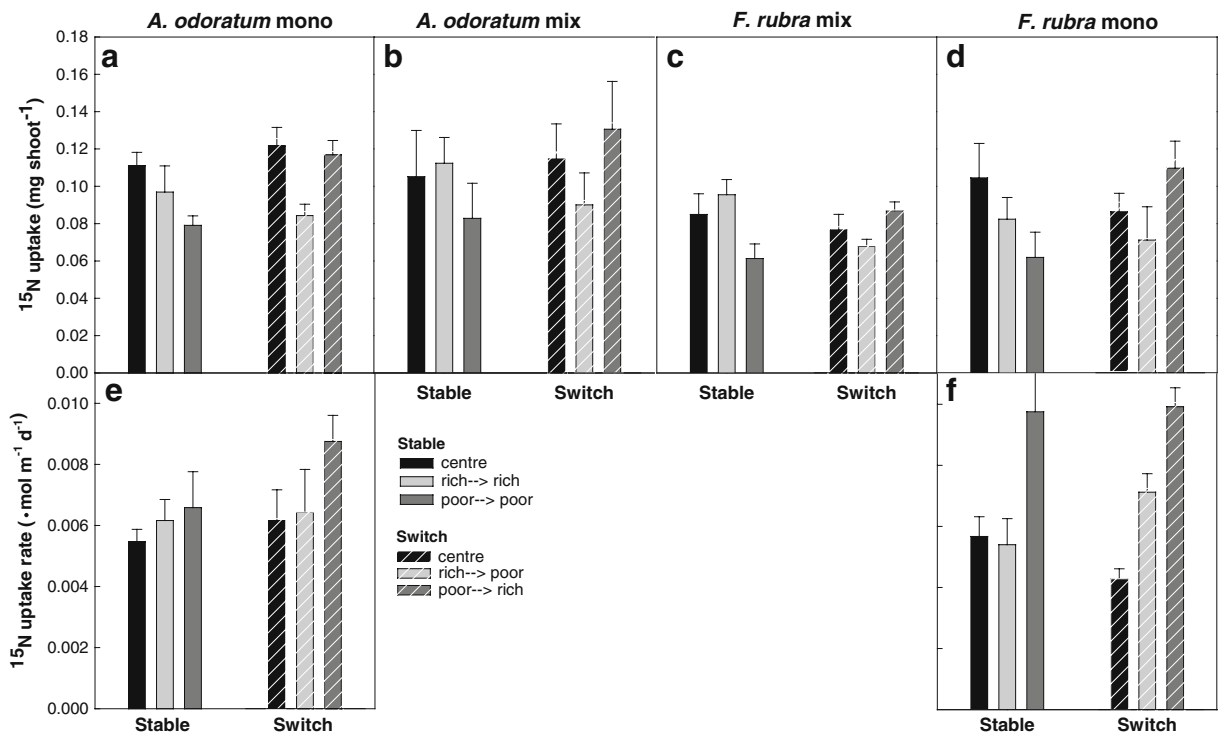


Fig. 3 ¹⁵N accumulation per plant (mg shoot⁻¹) of *A. odoratum* and *F. rubra* in monocultures (a,d) and mixture (b,c) in the different patches in the stable and switch treatment at final harvest. From the monocultures, ¹⁵N uptake per unit root length was calculated (μmol m⁻¹ d⁻¹). In mixture, *A. odoratum* takes

up more ¹⁵N than *F. rubra*. Nutrient uptake rate is dependent on patch type: In peripheral patches there is no species difference, in the centre patches uptake per unit root length is higher in *A. odoratum*. Values are means±SE, n=6

Table 2 ANOVA results (*F*-values) of ¹⁵N accumulation in the shoots (mg shoot⁻¹) of *A. odoratum* and *F. rubra*, grown in monoculture or mixture at final harvest

Source	¹⁵ N accumulation in shoot			
	Centre patch		Peripheral patches	
	<i>df</i>	<i>F</i> value	<i>df</i>	<i>F</i> value
Species	1	5.93*	1	8.23**
Planting	1	0.16 ^{ns}	1	0.93 ^{ns}
Patch type	1	0.02 ^{ns}	3	7.07***
Species × planting	1	1.06 ^{ns}	1	0.21 ^{ns}
Species × patch type	1	1.25 ^{ns}	3	0.10 ^{ns}
Planting × treat	1	0.07 ^{ns}	3	0.35 ^{ns}
Sp × plant × treat	1	0.05 ^{ns}	3	0.34 ^{ns}
Error (MS)	36	1.1 × 10 ⁻⁹	72	1.01 × 10 ⁻⁹

Analyses were performed separately for the centre patch peripheral patch types. n=6

P*<0.05, *P*<0.01, ****P*<0.001, *ns* non-significant

In general, both species took up similar amounts of ¹⁵N in monoculture and mixtures, although *F. rubra* had a non-significant trend towards a decreased uptake in mixture compared to monoculture. Unfortunately, we could not dissect the roots at the species level in the mixtures, so calculations regarding nutrient uptake rate in mixture were not possible.

¹⁵N uptake was strongly affected by patch type (Fig. 4; Table 3). ¹⁵N uptake from the centre patches was always among the highest of all patch types, but it was less than 30% higher than the uptake from the peripheral stable nutrient-rich patches, as would be expected on the basis of root length densities in these patches. The lowest ¹⁵N uptakes were achieved in the stable poor-poor patches with lowest root length densities.

Although the current experiment was not designed to measure physiological plasticity of the species, comparing the ¹⁵N uptake and root length densities in poor-rich (▼) patches versus poor-poor patches (◆) gives an indication about the contribution of morphological versus physiological plasticity for nutrient uptake in

Fig. 4 Relationship of **a** root length density (RLD) and **b** selectivity with ^{15}N accumulation in monocultures of *A. odoratum* and *F. rubra*. The range of produced root length densities, and thus the range of displayed selective root placement, in *F. rubra* is wider than that of *A. odoratum*. Still, *A. odoratum* takes up similar nutrients from peripheral patches and or more from centre patches. Closed symbols represent *A. odoratum*; open symbols represent *F. rubra*. Values are means \pm SE, $n=6$.

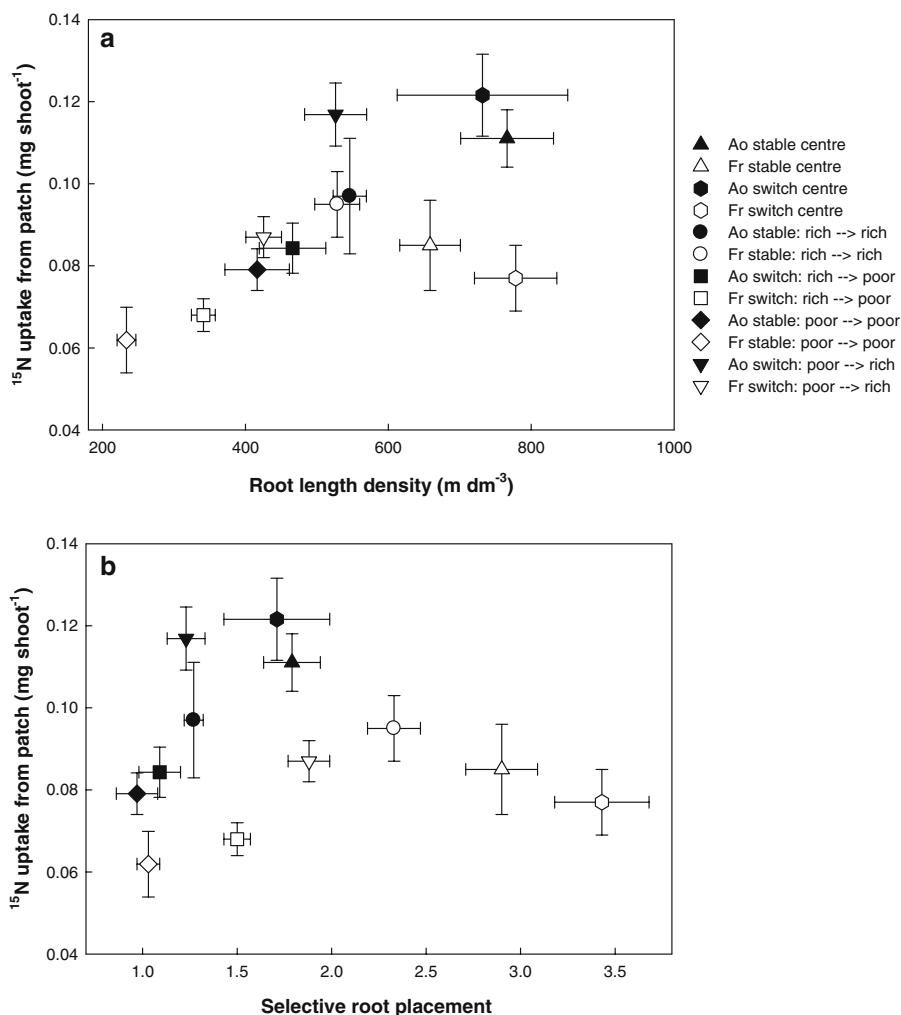


Table 3 ANOVA results (F values) of excess ^{15}N accumulation in the shoot (mg shoot^{-1}) and ^{15}N uptake rate per unit root length ($\mu\text{mol m}^{-1} \text{d}^{-1}$) of *A. odoratum* and *F. rubra* monocultures as a function of root length density (covariate factor) at final harvest

Source	^{15}N accumulation in shoot				^{15}N uptake rate of roots			
	Centre patch		Peripheral patches		Centre patch		Peripheral patches	
	df	F value	df	F value	df	F value	df	F value
Root length density	1	0.04 ^{ns}	1	0.816 ^{ns}				
Species	1	13.1 ^{**}	1	8.68 ^{**}	1	7.80*	1	0.63
Patch type	1	0.008 ^{ns}	3	5.82 ^{**}	1	0.01	1	1.46
Species*Patch type	1	0.984 ^{ns}	3	1.4 ^{ns}	1	3.46	3	0.82
Error (MS)	17	0.001	35	0.0001	18	0.002	36	0.004

Analyses were performed separately for the centre patch peripheral patch types. $n=6$

* $P<0.05$, ** $P<0.01$, ns non-significant

each of the two species (Fig. 4a). When exposed to a new nutrient-rich patch, *A. odoratum* achieved a relatively large increase in uptake rate with a modest increase in root length [difference in ^{15}N 0.0378 mg (+48 %); difference in RLD 110 m dm^{-3} (+26 %)]. *F. rubra* had a smaller increase in uptake rate with a larger increase in root length [(difference in ^{15}N 0.0251 mg (+41 %), difference in RLD 192 m dm^{-3} (+82 %)] in these newly enriched patches.

When ^{15}N uptake in the monocultures was correlated to selectivity (i.e. root length density in the target patch vs. root length density in the background soil; Fig. 4b),

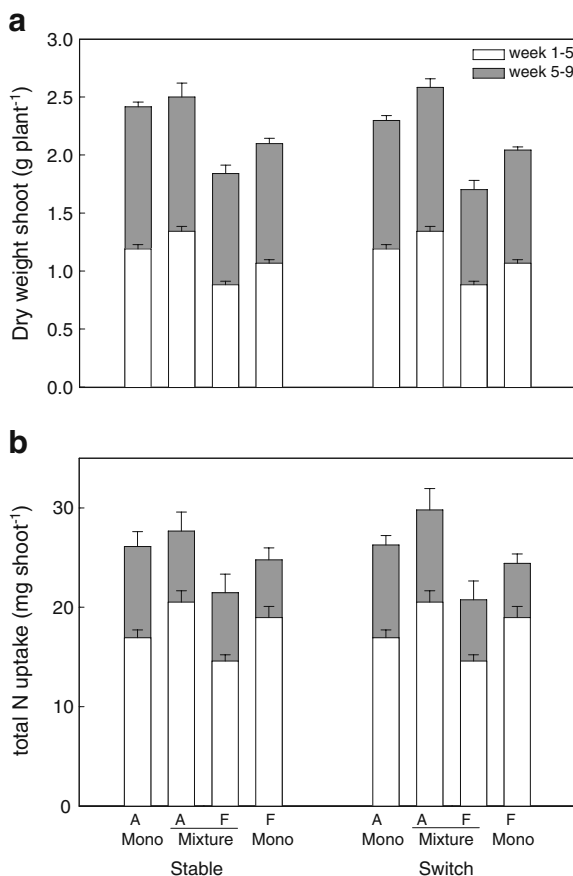


Fig. 5 Shoot biomass (a) and N accumulation (b) of *A. odoratum* and *F. rubra* in the stable and switch treatment in monocultures and mixtures at final harvest. *A. odoratum* is the stronger competitor of the two grasses. It increases shoot growth and N accumulation in mixture compared to monoculture at the expense of *F. rubra*. Growth in the first half of the experiment (until mid-harvest) is represented by the white bar, growth in the second half of the experiment by the grey bar. The total bar and error bars represent growth during the whole experiment. Values are means \pm SE. A = *A. odoratum* ($n=16$), F = *F. rubra* ($n=18$)

F. rubra appears to respond more by morphological changes to nutrient patches, as the range of selectivity of *F. rubra* is wider than that of *A. odoratum* without reaching the maximum N-uptake of the latter species.

Competitive hierarchy of the grass species

In monocultures, *A. odoratum* accumulated more shoot biomass than *F. rubra* (Fig. 5a; significant species effect; $F_{1,145}=24.3$, $p<0.001$; Table S1), despite its lower shoot biomass at the start of the experiment (0.026 ± 0.002 vs. 0.049 ± 0.003 g for *A. odoratum* and *F. rubra*, respectively; mean \pm SE). In mixtures, *A. odoratum* appeared to be the stronger competitor of the two species as shoot biomass of this species increased significantly under interspecific competition compared to its monoculture at the expense of shoot biomass production in *F. rubra*. Shoot biomass was not affected by the switch treatment (no treatment effect; $F_{1,145}=1.5$, $p>0.05$; Fig. 5a).

Plant growth was proportional to N accumulation, as biomass and N accumulation show similar patterns among treatment and species (Fig. 5b; Table S1). *A. odoratum* took up significantly more nitrogen than *F. rubra* (Fig. 5b; significant species effect; $F_{1,124}=7.2$, $p<0.001$) and tended to increase nutrient uptake in interspecific competition at the expense of *F. rubra*, but this was not significant (Fig. 5c; no species \times planting interaction; $F_{1,124}=2.4$, $p>0.05$). Again, no effect of switching nutrient patches was observed ($F_{1,124}=0.2$, $p>0.05$). Although shoots doubled in biomass in the second half of the experiment, total N uptake increased by only 30–50%. This corresponds with decreasing nitrogen contents in the second half of the experiment: N percentage in *A. odoratum* decreased from 1.6 to 1.1%, and *F. rubra* from 1.8 to 1.3% N of dry matter.

Discussion

We expected that the species with the highest selective root placement (*Festuca rubra*) would produce most roots in the nutrient-rich patch and take up most nutrients from it leading to competitive advantage over a species with less selective root placement (*Anthoxanthum odoratum*). We also expected that this advantage would decrease in a soil with switching nutrient patches.

Our results indeed marked *F. rubra* as the species that placed its roots much more selectively in nutrient-rich patches. However, in contrast to our expectations, both species achieved similar root length densities in the nutrient hotspots. Moreover, *A. odoratum* captured more nutrients from these patches than *F. rubra*. Thus, *A. odoratum* rather than *F. rubra* was the superior competitor, irrespective of the dynamics of the soil heterogeneity (i.e. nutrient switch treatment). Selective root placement did not appear to be an essential trait for high competitive ability in heterogeneous soils (Bliss et al. 2002; Cahill and Casper 1999; Fransen and de Kroon 2001; Rajaniemi 2007), not even in the short term. In contrast, achieving high root densities, but with less selectivity in root placement, appeared beneficial in both stable and dynamic heterogeneous conditions. A high specific root length and concomitant low investment in root mass seem essential components for this alternative competitive strategy.

Selectivity versus root length density: the role of specific root length

F. rubra allocated a larger amount of biomass to the nutrient patches than *A. odoratum*, as in Fransen et al. (2001, 1998), but this did not result in the highest root length density in the nutrient-rich patches, as these were similar between the species. Thus, in contrast to our first hypothesis, the higher selectivity of *F. rubra* in terms of biomass investment compared to *A. odoratum* did not lead to a proportionally increased root length density in a nutrient-rich patch. *A. odoratum* displayed a lower selectivity than did *F. rubra*, since relatively more of its roots were placed in the background soil. This higher background root length density of *A. odoratum* required lower biomass investments than the lower background root length density in *F. rubra*. The contrast between biomass investments and realised root length densities highlights the importance of the specific root length when studying root foraging responses. This conjectured role of specific root length in root foraging underscores the results of a multivariate analysis of (root) traits of 16 North American grassland species, which suggested that the biomass gain in heterogeneous versus homogeneous soils is more correlated to specific root length than to the selectivity (precision) of root placement (Kembel et al. 2008). Our data are consistent with this result, and

show that, although both these parameters may be important, their ultimate effects are probably mediated via increased root length density.

Differences in root foraging behaviour have long been interpreted as a trade-off between precision and scale (Campbell et al. 1991; Farley and Fitter 1999; Wijesinghe et al. 2001; Rajaniemi and Reynolds 2004), with dominant species employing low-precision foraging at high scale and subordinate species vice versa. It has been argued (Kembel and Cahill 2005; Kembel et al. 2008; de Kroon and Mommer 2006) that the concept of 'scale' as the spatial extent or size of the root system was imprecisely defined, but 'scale' may also be interpreted as root densities per unit soil volume. If defined in this way reminiscent of Campbell et al. (1991), our results suggest that high 'scale' foraging (i.e. high root length densities of *A. odoratum*) can lead to effective nutrient capture and competitive ability as an alternative to foraging with more 'precision' (*F. rubra*) even in a stable patchy environment. A high specific root length seems a prerequisite for this to happen as roots have to be sufficiently cheap in terms of biomass investment.

The functionality of root production: nutrient uptake

As formulated in our second hypothesis, nutrient uptake from a particular patch was proportional to the root length density achieved. *A. odoratum* achieved more root length, and accumulated more ¹⁵N uptake from the different patches than did *F. rubra*. Nutrient uptake rate per unit root length was similar between the species in the peripheral patches, but not in the centre patches. In a previous study (Hodge et al. 1999), two competing grass species also had similar ¹⁵N uptake rates per unit root length. In the centre patches, *F. rubra* showed increased root investment compared to the peripheral patches, but this did not lead to more nutrient uptake, suggesting root overproduction in the larger patches with intense competition (see Fransen and de Kroon 2001; Gersani et al. 1998; Mommer et al. 2010). *A. odoratum* plants were positioned similarly within the pots as *F. rubra*, but showed a different response: continuously increased nutrient uptake with root length density.

Unlike our third hypothesis, nutrient uptake and biomass production of *F. rubra* were not affected by increasing patch stability, and neither was *A. odoratum*. This may be due to the fact that even in the switch

conditions a large part (i.e. half) of the nutrients was permanently available in the centre patches. Another explanation may be that the nutrients in the permanent patches were taken up within two days, i.e. before the next application of the nutrients took place, indicating that “permanent” may not have been perceived so by the plants. Wijesinghe et al. (2005) also observed significant effects of patch longevity on root biomass, but hardly on shoot biomass production in a community setting—even on a longer time scale. Moreover, Jansen et al. (2006) showed that plants that experienced shifting short-lived nutrient patches achieved similar biomass and N uptake as plants grown under stable heterogeneous conditions, whereas the root distribution patterns were completely different. Patch dynamics, simulated by switching the patches halfway the experiment affected root distribution belowground significantly, but had no consequences for growth aboveground. Morphological responses, probably combined with rapid physiological changes seem to result in an apparent homeostasis aboveground, as also observed in other studies (Jansen et al 2006; Wang et al. 2009).

As said above, the strategy of *A. odoratum*—producing high root length densities at low biomass investment—was a beneficial strategy in our experiment. Interestingly, in a 2-year container experiment using the same two grasses (Fransen et al. 2001), *F. rubra* appeared to be the better competitor of the two species. After one growing season, *F. rubra* achieved higher shoot biomass in mixture at the expense of *A. odoratum* in both homogeneous and heterogeneous soil conditions. This competitive advantage of *F. rubra* in mixtures was most likely due to increased biomass investments of this species belowground as *F. rubra* in monocultures produced twice as much root biomass as *A. odoratum*. However, competitive hierarchy changed in the second growing season, when nutrients became increasingly limiting and the root investments of *F. rubra* appeared too costly relative to the benefits in terms of nutrient uptake. Comparing the experiments of Fransen et al. (2001) with ours suggests the significance of the overall nutrient content and/or the continuous supply of nutrients via mineralization. *F. rubra* seems the stronger competitor of the two species if nutrients are sufficiently available, since then the (over)production of roots pay back. If the system is poorer in nutrients—as it most probably was in our experiment on sand mixed with vermiculite and N-solution—*A.*

odoratum is not outcompeted by *F. rubra*, and may in fact even win competition in heterogeneous soils. This suggests that having roots with a high specific root length can be an alternative strategy under heterogeneous nutrient conditions.

Conclusion

F. rubra showed the highest selective root placement of the two investigated grass species, but did not win the competition. *A. odoratum* was the stronger competitor, produced overall higher root length densities and realized higher nutrient uptakes than *F. rubra*. This strategy appeared beneficial since biomass investments of the roots of *A. odoratum* were lower compared to *F. rubra*, as the specific root length was 30% higher in the first species. Placing roots selectively in nutrient hotspots may thus be one way to achieve high nutrient uptake, but, alternatively, producing high root length densities at little biomass costs can be similarly functional. If this alternative strategy appears to be general to more species than just *A. odoratum*, it may explain the general lack of correlation between selective root placement and growth in heterogeneous versus homogeneous conditions (Kembel and Cahill 2005; Kembel et al. 2008).

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