Stellingen

I. Wortelonderzoek kan het daglicht niet verdragen.

II. De algemeen gangbare opvatting dat snelgroeiende soorten morfologisch plastischer zijn dan langzaamgroeiende soorten wordt niet ondersteund door de tot nu toe uitgevoerde foerageer-experimenten.
Dit proefschrift

III. De korte-termijn voordelen van selectieve wortelplaatsing worden op de lange-termijn te niet gedaan door nutriëntenverliezen als gevolg van het afsterven van wortels na uitputting van nutriëntenrijke plekken.
Dit proefschrift

IV. Verschillen in foerageermechanismen tussen plantensoorten resulteren in een verschuiving van het concurrentievermogen in heterogene standplaatsen ten opzichte van homogene standplaatsen, zelfs als de totale hoeveelheid beschikbare nutriënten in beide standplaatsen gelijk is.
Dit proefschrift

V. Dichtheidsafhankelijke sterfte als gevolg van concurrentie tussen planten zal in natuurlijke vegetaties slechts zelden leiden tot een regelmatig patroon.

VI. Een afstudeervak waarin begrazing moet worden gesimuleerd trekt meer studenten dan een afstudeervak waarin planten regelmatig moeten worden geknipt, terwijl de werkzaamheden identiek zijn.

VII. Om de afname van het aantal sollicitanten op een promotieplaats te stoppen is het bieden van laptops, fietsen en bonussen niet voldoende. Talentvolle onderzoekers hebben meer behoefte aan loopbaanmogelijkheden op de universiteit (De Volkskrant, d.d. 17 juli 1999).

VIII. Academici liegen vaker dan anderen (Gelders Dagblad, d.d. 3 mei 1999), waaruit blijkt dat wijsheid en waarheid niet onlosmakelijk met elkaar verbonden zijn, hetgeen ook blijkt uit de beide gezegdes: "In Vino Veritas" (Horatius) en "Als de wijn gaet in den man, leyt de wijsheyt in de kan" (Jacob Cats).

IX. Om gelijke tred te houden met de verengelsing in functieomschrijvingen zou een kas- en proefveldbeheerder zich tegenwoordig beter plant manager kunnen noemen.

X. Als je omslaat met een kano, gaat de peddel voor het meisje.

Stellingen behorende bij het proefschrift: "Root foraging: the consequences for nutrient acquisition and competition in heterogeneous environments" van Bart Fransen.

Wageningen, 10 september 1999.

Root foraging

The consequences for nutrient acquisition and competition in heterogeneous environments

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Root foraging

The consequences for nutrient acquisition and competition in heterogeneous environments

Bart Leonardus Lambert Fransen

Proefschrift
ter verkrijging van de graad van doctor
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Abstract

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In natural habitats, the availability of essential mineral nutrients may vary widely from place to place and from time to time, at scales relevant to individual plants. Plants have developed root foraging mechanisms that enable them to acquire adequate amounts of nutrients in these heterogeneous environments. The ability of plants to proliferate roots in nutrient-rich patches has been shown frequently, but both the timing and the degree of root proliferation varied widely. Species from inherently nutrient-rich habitats in general display a higher relative increase in root density in nutrient-rich patches than species from inherently nutrient-poor habitats. This observation prompted the hypothesis that root foraging mechanisms differ between species from habitats of different nutrient availability.

Overall, the results described in this thesis contradict this hypothesis. The higher degree of selective root placement displayed by species from more nutrient-rich habitats compared to species from more nutrient-poor habitats may result from differences in growth rate rather than from differences in root morphological plasticity. The results further indicate that selective root placement may confer an advantage in terms of nutrient acquisition in heterogeneous environments in the short-term, but in the long-term the increased root density may result in a lower rather than a higher biomass production in heterogeneous environments. However, root foraging abilities by which local nutrient patches are exploited may still be profitable when plants are grown in competition. The ability to rapidly exploit nutrient-rich patches due to root foraging characteristics seems to confer a competitive advantage in heterogeneous environments, even in the long-term.

Keywords: competition, foraging, heterogeneity, nutrient uptake, perennial grasses, plasticity, root proliferation.

Contents

Chapter 1

General introduction

Plants are sometimes seen as dull because they 'do not move', they 'do not behave' and they seem altogether passive. This is all a gross illusion. As we shall see, plants are exciting if only you are perceptive enough to appreciate the subtleties of their peculiar ways.

J.W. Silvertown & J. Lovett Doust,
Introduction to plant population biology, 1993.

All plants require the same essential resources, such as light, water and nutrients, for maintenance, growth and reproduction. These resources are rarely evenly distributed within plant communities. An obvious example is the uneven distribution of light in the forest understorey, characterised by the constantly changing pattern of light flecks throughout the vegetation and on the ground. Perhaps less obvious is the uneven distribution of nutrients in soils. Soil patches of different nutrient availability are formed at various scales by abiotic factors (e.g. soil type, soil depth, micro-topography) as well as by biotic factors (e.g. litter production and decomposition).

The lives of plants are strongly influenced by their sessile nature and having to endure their local situation without being able to seek more favourable conditions (Bradshaw, 1965 in Bell and Lechowicz, 1994). However, individual plants are capable of placing leaves and root tips selectively in the resource-rich patches within their environment. These so-called foraging mechanisms enable plants to acquire adequate amounts of resources within their profoundly heterogeneous environments.

This thesis describes experiments that investigate the root foraging characteristics of species of habitats that differ in nutrient availability in order to assess their long-term consequences for nutrient acquisition and competitive ability in heterogeneous environments.

Nutrient heterogeneity

Heterogeneity refers to a non-uniform distribution of resources or other biotic and abiotic environmental conditions in the natural surrounding of an organism (Stuefer, 1996). Nutrient availability may vary considerably within habitats, both in space and time (Robertson *et al.*, 1988; Lechowicz and Bell, 1991; Gross *et al.*, 1995; Miller *et al.*, 1995; Ryel *et al.*, 1996; Cain *et al.*, 1999), and even within the vicinity of individual plants. For example, in cold desert, nitrate concentration in the soil solution varied by an average factor of 12 at a 12.5cm scale and even at a scale of 3cm by an average factor of 2.8 (Jackson and Caldwell, 1993). Such small-scale heterogeneity can have profound effects on the performance of individual plants and on plant population dynamics (Antonovics *et al.*, 1987; Bell and Lechowicz, 1991; Bell *et al.*, 1991; Miller *et al.*, 1995; Reynolds *et al.*, 1997).

It should be noted that heterogeneity is a general term that comprises several aspects such as contrast, scale, aggregation, predictability and spatial co-variance. Below I will

address only a few of these aspects; for elaborated treatments of heterogeneity see for example, Kotliar and Wiens (1990), Li and Reynolds (1995) and Stuefer (1996).

Contrast refers to the degree of difference between patches or between the patch and the surrounding matrix (Kotliar and Wiens, 1990). If contrast is absent within species-specific perception limits, plants perceive their environment as functionally homogeneous (Stuefer, 1996). The level of contrast that is necessary to induce a response is species- and resource-dependent (Stuefer, 1996).

Scale refers to the spatial and temporal dimensions of patches in a heterogeneous environment. Plants can perceive spatial heterogeneity only within a certain range of scales (Stuefer, 1996). The smallest scale at which an organism (e.g. plant) is able to respond to heterogeneity is termed 'grain' (Kotliar and Wiens, 1990). At smaller scales, the organism functionally perceives its environment as homogenous and does not respond to any structure that might actually exist (Kolasa 1989). The largest scale of heterogeneity to which an organism can respond is termed 'extent' (Kotliar and Wiens, 1990). The same terms can be used with regard to temporal heterogeneity. The range of temporal scales (i.e. patch longevity) that can be perceived and responded to by plants is determined by the response time of the induced processes (i.e. temporal grain; Stuefer, 1996), and by the lifetime of the organism (i.e. temporal extent; Stuefer, 1996). For example, in response to nutrient patches that are short-lived, 'slow' morphological plant responses like root proliferation are unlikely to enhance nutrient acquisition, but, in contrast, 'fast' physiological response, such as changes in the uptake capacity of roots, may increase nutrient uptake by plants (De Kroon and Schieving, 1990).

The recurrent view in the literature on nutrient heterogeneity in different habitats is that spatial heterogeneity in nutrient availability is more marked in inherently nutrient-rich habitats whereas temporal nutrient heterogeneity is more important in inherently nutrient-poor habitats. This view originated from the influential paper of Chapin (1980) on the mineral nutrition of wild plants. Chapin (1980) stated, based on his own work in tundra's (Chapin and Bloom, 1976; Chapin *et al.*, 1978), that 'in infertile habitats it is likely that a large percentage of annual nutrient absorption occurs during nutrient flushes, particularly during late winter and early spring, rather than by steady-state absorption under average conditions'. While perhaps valid only for extremely nutrient-poor habitats, such as tundra's, Grime et al. (1986) generalised this view by stating in their paper on the ecological significance of plasticity that 'These (i.e. reversible physiological changes) (...) facilitate the exploitation of the pulses of temporary and unpredictable resources supply which are characteristic of unproductive

habitats'. Hereafter the statement that the supply of nutrients in nutrient-poor habitats is restricted to short, unpredictable nutrient pulses reappeared in numerous papers (Crick and Grime, 1987; Campbell and Grime, 1989; Hutchings and De Kroon, 1994).

To date, however, there are no published studies that show conclusively that nutrient-poor habitats differ in variability of nutrient concentration than nutrient-rich habitats (Robinson and Van Vuuren, 1998). Until now only two studies (Ryel *et al.*, 1996; and Cain *et al.*, 1999) have examined both spatial and temporal variation in nutrient availability during a growing season, but unfortunately they did not compare habitats that differed in nutrient availability. The only certainty about differences between nutrient-poor and nutrient-rich habitats is that they differ in the overall level of nutrient supply. In general, nutrient supply will largely depend on the processes of decomposition and mineralisation, which themselves are a function of temperature, soil moisture and soil acidity (Berendse *et al.*, 1994).

Foraging

Plants have developed mechanisms that enable them to acquire adequate amounts of essential resources in heterogeneous environments. The description of plant responses to environmental heterogeneity in terms of foraging was first used by Bray (1954) when he described the search patterns of roots for nutrients in the soil. The term foraging has become common usage in plant ecology through the work of Grime and co-workers who, in analogy with the acquisition of patchily distributed food sources in animals, used 'foraging' to describe the ability of plants to project leaves and roots in resource-rich patches within the environment (Grime, 1979; Grime *et al.*, 1986; Campbell *et al.*, 1991).

Foraging is defined as the processes whereby an organism searches, or ramifies within its habitat, which enhance its acquisition of essential resources (Hutchings and De Kroon, 1994; De Kroon and Hutchings, 1995). Foraging in plants is accomplished by morphological plasticity in response to environmental conditions, and may result in the selective placement of resource acquiring structures (leaves and roots) within the environment (Grime *et al.*, 1986; Hutchings and Slade, 1988; Hutchings and De Kroon, 1994; De Kroon and Hutchings, 1995; Oborny and Cain, 1997). Plasticity is shown by a genotype when its expression can be altered by environmental influences. The change that occurs can be termed the response. Since all

changes in the characters of an organism that are not genetic are environmental, plasticity is applicable to all intra-genotypic variability (Bradshaw, 1965).

Plants have frequently shown to be able to alter root morphology in response to nutrient enrichment, resulting in the proliferation of roots in nutrient-rich patches (Drew *et al.*, 1973; Drew, 1975; Drew and Saker 1975; Drew and Saker 1978; Crick and Grime, 1989; Granato and Raper, 1989; Jackson and Caldwell, 1989; Gross *et al.*, 1993; Pregitzer *et al.*, 1993; Larigauderie and Richards, 1994; Bilbrough and Caldwell, 1995). However, both timing and degree of root proliferation appears to be highly variable among species from different habitats.

In his triangular model of primary plant strategies, Grime (1974, 1979) proposed that there are three extremes of ecological specialisation (i.e. competitors, stress-tolerators and ruderals) each characterised by a set of traits within which distinct forms of plasticity are of major importance. The most interesting implication concerns the difference in the method of resource capture exhibited by the competitors and the stress-tolerators. (Grime, 1979).

Competitors are species characteristic of stable productive habitats that depend upon the ability to sustain high rates of resource capture above and below ground. Morphological plasticity in the development of shoot and roots, together with the continuous repositioning of leaves and roots, brings about a continuous adjustment in the spatial distribution of absorptive surfaces above and below ground. Plasticity in competitors is part of an 'active foraging mechanism whereby high rates of resource capture are achieved through the ability to locate functional leaves and roots in the resource-rich zone's (Grime *et al.*, 1991).

Stress-tolerators are species characteristic of unproductive habitats that depend primarily upon the capacity to capture and retain scarce resources. The leaves and roots of stress-tolerators will be comparatively long-lived structures in which plasticity is expressed mainly through reversible physiological changes, which maintain functional integrity over the long life spans of individual organs and facilitate exploitation of resource pulses (e.g. mineralisation from decomposition events, Grime *et al.*, 1991).

Evidence is accumulating that inherently fast-growing species from nutrient-rich habitats display a higher degree of root morphological plasticity in response to nutrient enrichment than inherently slow-growing species from nutrient-poor habitats. Several studies have shown that fast-growing species generate larger relative differences in root length or root biomass per unit soil volume between nutrient-rich and nutrient poor patches (Crick and Grime, 1987; Caldwell *et al.*, 1991; Robinson and Van Vuuren, 1998).

This general observation raises the question to what extent the differences in foraging ability between fast- and slow-growing species result from differences in morphological plasticity or from differences in growth rate.

It is important to distinguish foraging from growth. Foraging precedes and enhances resource uptake whereas growth follows from resource uptake (Hutchings and De Kroon, 1994). However, a major problem exists with distinguishing foraging from growth, because some morphological alterations that accomplish the foraging responses to enhanced resource supply are expected simply as a result of the enhanced growth rate that is achieved when more resources are available. Hutchings and De Kroon (1994) suggested a "null-model" of foraging in which resource availability affects only the growth of the plant. A higher growth rate may be realised by a higher rate at which new stem and root branches and internodes are produced, as well as by the formation of longer and thicker branches. Common root morphological responses such as enhanced root length growth rate and enhanced lateral root branching accord with this null-model and should be regarded as manifestations of growth (Hutchings and De Kroon, 1994). Viewed in this way, root foraging ability and growth rate may be two sides of the same coin, and this may explain why fast-growing species display a more effective foraging behaviour than slow-growing species.

In most empirical studies, morphological plasticity is typically analysed at a common point in time (Coleman *et al.*, 1994; but see Rice and Bazzaz 1989 for a notable exception). The length of the growth period is identical for each species even though the inherent growth rate of the species is different. However, plants growing with different rates will be of different sizes when compared at a common point in time and may have different patterns of biomass partitioning over the various plant parts (Evans, 1972; Coleman *et al.*, 1994; Coleman and McConnaughay, 1995). However, if plants follow the same developmental trajectory, there may be no differences in biomass partitioning when these plants are compared at equal sizes. Plasticity in traits representing any aspect of plant biomass should therefore be examined as a function of common biomass (Evans, 1972). Because foraging is accomplished by morphological plasticity, differences in foraging ability between species need to be examined at a common size instead of at a common time (see Hutchings & de Kroon 1994). Hence, while empirical evidence suggests that faster-growing species are more morphologically plastic than slower-growing species, this conclusion cannot be derived with certainty because of the interaction between growth rate and morphological plasticity.

Assessing the ecological significance of root foraging for mature perennial species in heterogeneous habitats

According to foraging theory (Stephens and Krebs, 1986; Hutchings and De Kroon, 1994), selection in natural habitats will favour the foraging behaviour that generates the highest net long-term resource acquisition.

In spite of the numerous studies on root morphological plasticity, still very little is known about the long-term benefits in terms of nutrient acquisition of root foraging responses. Many root foraging experiments are carried out with young plants, often seedlings, over a short time span (typically in the order of weeks), showing high rewards in terms of nutrient acquisition of root proliferation in heterogeneous environments (Drew, 1975; Drew and Saker, 1975; Drew and Saker, 1978; Crick and Grime, 1987; Granato and Raper, 1989). However, there are reasons to assume that the rewards in terms of nutrient acquisition of root proliferation for larger perennial plants in natural habitats may be lower than expected based on root foraging studies carried out so far.

Firstly, trade-offs between investments in foraging structures and other plant functions (e.g. storage and reproduction) may play a role. Active foraging for immediate returns may comprise the long-term nutrient acquisition if reduced growth and storage reduces the future performance of the plants. However, trade-offs with reproductive or storage functions will not be manifested until the perennial plants reach some mature state.

Secondly, in many root foraging studies, the nutrient concentration in the enriched patches is kept constant, due to a continuous replenishment with nutrient solution during the experiment (Drew, 1975; Drew and Saker, 1975, 1978; Crick and Grime, 1987; Granato and Raper, 1989;, Campbell and Grime, 1991). However, in natural habitats patch depletion occurs due to leaching nutrient uptake by plants and micro-organisms. Patch depletion is shown to limit the profits of root proliferation, (Van Vuuren *et al.,* 1996; Hodge *et al.,* 1998), and this may become more pronounced in the longer run.

Thirdly, all root foraging studies so far were run over too short periods to include effects of root turnover. The merits of root foraging in response to nutrient heterogeneity are often defined in terms of nutrient uptake, but the net long-term nutrient acquisition of perennial plants is dependent on the balance between nutrient uptake and nutrient losses due to turnover of plant parts (Berendse 1985; Berendse 1994a,b). This balance can, however, only be accessed in long-term experiments with large, mature plants.

Apart from these reasons why the benefits of selective root proliferation may be less advantageous than appears from experiments carried out today, the ecological significance of root proliferation, particularly in response to nitrate-enriched patches has been obscure for a long-time. Root proliferation enhances the uptake of poorly mobile nutrients such as phosphate. Most phosphate acquired by a plant originates in soil less than 1mm from the root surface (Nye and Tinker, 1977). Nitrate, in contrast, diffuses some three to four orders of magnitude faster than phosphate, and to absorb all nitrate from a patch roots should not have to proliferate as much as in a phosphate patch. However, species display similar degrees of root proliferation in response to nitrate-enriched patches as to phosphate enriched patches (Drew *et al.*, 1973; Drew and Saker, 1978; Robinson, 1996). Combined with the above mentioned reasons, all limiting the rewards, in terms of nutrient acquisition, of root proliferation, the question arises why root proliferation in response to nutrient enrichment is so widespread among plants species. As a possible answer to this question, it has recently been suggested that root proliferation may confer a competitive advantage (Robinson *et al.*, 1999). The ability to rapidly reach, fill and deplete nutrient-rich patches prior to neighbouring plants will enhance to nutrient capture in a competitive environment.

The species

Comparative studies that tested for differences in root foraging ability between species have used species that differed widely in relative growth rate, thereby including species of different families and growth-form (e.g. Crick and Grime, 1987; Campbell *et al.*, 1991). However, root architecture may in part be phylogenetically determined (Fitter and Stickland, 1991). Much of the variation between species may be associated with phylogenetic constrains. For example, the tendency for the grasses to exhibit less precise foraging than the herbs in the experiment of Campbell *et al.*, (1991) may in part be phylogenetically determined (Grime, 1994). Hence, to avoid confounding effects of gross differences in growth form and phylogeny in the analysis of root foraging ability, species of a single family (Gramineae) were used this thesis.

The consequences of root foraging for the nutrient acquisition and competitive ability of species in this thesis are investigated using *Lolium perenne* L., *Holcus lanatus* L., *Festuca rubra* L., *Anthoxanthum odoratum* L. and *Nardus stricta* L., all common perennial grasses with a wide distribution in western Europe (Weeda, 1994). The species used originate from

different fields along the Anlooër diepje, a brook in the 'Drentsche Aa' Nature Reserve in The Netherlands. The management in these former agricultural grasslands changed from cutting twice a year with fertilisation to cutting once a year without fertilisation (see Bakker, 1989). The fields differ in nutrient availability because the application of fertiliser was stopped in different years (Olff *et al.*, 1990). The annual removal of the organic matter after mowing resulted in a marked decline in mineralisation and productivity, and in concomitant changes in the species composition (Olff and Bakker 1991; Olff *et al.*, 1994). The pasture species *Lolium perenne* L. is replaced by *Holcus lanatus* L. shortly after fertilisation stopped. *Holcus lanatus* L. in turn is gradually replaced by *Festuca rubra* L. and *Anthoxanthum odoratum* L. (Olff *et al.*, 1990; Olff and Bakker 1991). The last species, *Nardus stricta*, occurs only in the most nutrient-poor fields along the Anlooër diepje (Bakker 1989).

Aim and outline of this thesis

The central aim of this thesis is to answer the question: 'Do species from habitats that differ in nutrient availability utilise different foraging mechanisms to acquire heterogeneously distributed soil resources, and do these foraging characteristics contribute to the success of the species in their indigenous habitats?'

To answer whether species from habitats of different nutrient availability differ in root foraging mechanisms, we first have to make an unambiguous distinction between foraging and growth rate, because phenotypic variation between these species may result from differences in growth rate rather than from differences in foraging ability. In Chapter 2, we show theoretically how the effects of foraging and growth rate on root biomass production in response to heterogeneity can be disentangled.

The first experimental chapter (Ch. 3) describes the short-term root morphological and physiological responses of the species in response to spatial and temporal nutrient heterogeneity. Nutrient heterogeneity is created by applying equal amounts of nutrient solution in different spatial and temporal patterns. The ability of the species to acquire nutrients from temporally enriched nutrient patches is compared with their ability to exploit spatially enriched patches. This experiment provides basic information on root morphological and physiological plasticity of the species.

In Chapter 4, we study the longer-term consequences of root foraging ability of the species in response to spatial nutrient heterogeneity. To mimic natural habitats, nutrient heterogeneity is created by mixing soils of different nutrient availability allowing patch depletion over a period of 3 months. The effectiveness of the root responses in terms of nutrient acquisition is determined by comparing the amount of nitrate and phosphate captured by the species in the heterogeneous treatment with that in a homogeneous treatment that had the same overall nutrient availability.

In Chapter 5, the long-term effects of differences in root foraging ability and root turnover between *Holcus lanatus* a species characteristic of nutrient-rich habitats and *Nardus stricta* a species from nutrient-poor habitats on biomass production are determined during a two year experiment. Minirhizotrons were used to assess the root dynamics of the species non-destructively. In this way, the effects of differences in root turnover, primarily occurring during winter, on the effectiveness to exploit nutrient-rich patches can be studied. The species were grown under two levels of overall nutrient availability, but the contrast between the nutrient-rich and nutrient-poor patch was the same under both overall levels of nutrient availability. The same contrast under both overall levels of nutrient availability may invoke equal root foraging responses, but the benefits of root foraging may be lower in the overall low level of nutrient availability, because plants may not be able to acquire sufficient nutrients to offset their nutrient losses due to root turnover.

In Chapter 6, we investigate the effects of differences in root foraging ability and nutrient acquisition on the competitive ability of species in heterogeneous habitats in another two-year experiment. *Festuca rubra* and *Anthoxanthum odoratum*, two species with similar growth rates were used to avoid differences in competitive ability resulting from large differences in plant size between the species. The species were grown in monocultures and mixtures in homogeneous and heterogeneous treatments. Nutrient heterogeneity was introduced at two spatial scales, coarse- and fine-grained, but the overall level of nutrient availability was the same in all treatments. Strontium labelling was used to determine the ability of the species to acquire nutrients when grown in mixtures in homogeneous and heterogeneous environments.

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Chapter 2

Disentangling the effects of root foraging and inherent growth rate on plant biomass accumulation in heterogeneous environments: a modelling study

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Abstract

Empirical evidence indicates that fast-growing species generally display a higher degree of selective root placement in heterogeneous environments than slow-growing species. Such root foraging is accomplished by root morphological responses, but since some morphological responses are simply the result of enhanced growth of the roots in the enriched patch it is difficult to separate the effects of root foraging and growth rate on the biomass accumulation of species in heterogeneous environments.

Here a simple model is presented to disentangle these effects of root foraging and relative growth rate. Root foraging is incorporated as the selective allocation of root biomass per unit time to the nitrogen-rich patch. Growth rate differences among the model plants result from differences in nitrogen utilisation efficiency. In the model, the degree of selective root placement can be varied independently of growth rate.

The model shows that when plants are compared at a common point in time, selective root placement and growth rate interact positively with respect to the enhancement of plant biomass accumulation in heterogeneous compared to homogeneous environments. However, by evaluating the model at a common plant biomass, the main and interactive effects of growth rate are eliminated. These results suggest that growth rate by itself does not confer an advantage in terms of resource acquisition and biomass accumulation in heterogeneous environments. Only the selective placement of resource acquiring structures (such as roots) leads to such benefits. The essential differences between foraging and growth, as well as the consequences of differences in foraging ability and growth rate between species for the competition for a limited resource, are discussed.

Keywords: environmental heterogeneity, foraging, growth rate, model, nitrogen uptake, nitrogen utilisation, patchiness, plant growth, plasticity, root placement

Introduction

Resources that are essential for plant growth (e.g. light and nutrients) are non-uniformly distributed within the neighbourhood of the plant (Jackson and Caldwell, 1993; Stark, 1994). Morphological plasticity enables plants to generate different patterns of placement of resource-acquiring structures in response to different environmental conditions, thereby enhancing the acquisition of essential resources - a process referred to as the foraging ability of plants (Hutchings and De Kroon, 1994; De Kroon and Hutchings, 1995).

Root morphological plasticity generates higher root length and root biomass per unit soil volume in nutrient-rich patches compared to nutrient-poor patches (see Robinson, 1994; Robinson and Van Vuuren, 1998). In general, fast-growing species display a higher degree of root morphological plasticity than slow-growing species. Several studies show that fast-growing species generate larger relative differences in root length or root biomass per unit soil volume between nutrient-enriched and nutrient-poor patches than slow-growing species (Crick and Grime, 1987; Fransen *et al.*, 1998; Robinson and Van Vuuren, 1998).

This general observation raises the question to what extent the differences in root density responses between species result from differences in foraging ability or from differences in growth rate. Two main problems exist with distinguishing foraging from growth in this context.

First, some morphological responses to enhanced resource supply are expected simply as a result of the enhanced growth rate that is achieved when more resources are available. Hutchings and De Kroon (1994) suggested a "null-model" of foraging in which resource availability affects only the growth of the plant. A higher growth rate may be realised by a higher rate at which new stem and root branches and internodes are produced, as well as by the formation of longer and thicker branches. Common root morphological responses such as enhanced root length growth rate and enhanced lateral root branching accord with this null-model and should be regarded as manifestations of growth (Hutchings and De Kroon, 1994). Viewed in this way, root foraging ability and growth rate may be two sides of the same coin, and this may explain why fast-growing species display a more effective foraging behaviour than slow-growing species.

Second, growth rate may also play an important role with respect to the distribution of root length and root biomass per unit soil volume among nutrient-enriched and nutrient-poor

patches within the rooting volume. Let us assume that fast- and slow-growing species are equally selective in root placement, i.e. they allocate a similar proportion of root biomass to rich vs. poor patches per unit of time. After a given period of time, fast-growing species will then have produced more root length and root biomass in the nutrient-rich patch than slow-growing species, simply as a result of their higher growth rate. Since nutrient acquisition will especially depend on the amount of roots in the nutrient-rich patch, fast-growing species may be expected to acquire more nutrients than slow-growing species in heterogeneous environments compared to homogeneous environments, even though the degree of selective root placement is the same.

To disentangle the effects of foraging and growth rate on nutrient acquisition we developed a simple analytical model of whole plant biomass accumulation, using nitrogen as an example for nutrients. In the model, differences in relative growth rate between species are assumed to result from differences in nitrogen utilisation efficiency, i.e. the amount of biomass produced per unit of acquired nitrogen (Hunt *et al.*, 1990). Selective root placement is accomplished by a higher root biomass production per unit of time in the nitrogen-rich vs. the nitrogen-poor patch that, in turn, is the result of morphological responses such as enhanced root branching and root length growth in the richer patch. At the whole plant level and in the model, selective root placement is expressed as a higher percentage allocation of newly produced biomass to the nitrogen-rich patch per unit of time compared to the nitrogen-poor patch.

In this way, growth rate and selective root placement can be varied independently in the model. Their effects are assessed by comparing the biomass accumulation of plants as a function of relative growth rate and as a function of the degree of selective root placement. Whole plant biomass in heterogeneous environments is evaluated both after a given period of time as is done in most empirical studies, but also at a common whole plant biomass as is recommended by Coleman *et al.* (1994) for studies of biomass allocation and resource acquisition. The model is used to answer the following questions: What are the effects of selective root placement and growth rate on plant biomass accumulation in heterogeneous environments, and do selective root placement and growth rate interact in their effects?

In the model a few deliberately simple assumptions are made. The nitrogen supply in both the nitrogen-rich and nitrogen-poor patch is kept constant, and nitrogen uptake is assumed to be proportional to root biomass. Under these conditions, plants will continue to

grow exponentially and selective root placement will have its maximum returns because the nutrients in the patches remain undepleted. While unrealistic, under these assumptions the model will show the greatest effects of relative growth rate and selective root placement on plant biomass accumulation in heterogeneous environments.

Model description

Plant growth

Total plant biomass (i.e. dry weight) (M_T) is given by:

$$M_T = M_L + M_{Rr} + M_{Rp} \quad (1)$$

M_L, M_{Rr}, and M_{Rp} are the biomass of leaves, roots in the nitrogen-rich patch, and roots in the nitrogen-poor patch, respectively.

Plant growth, i.e. biomass accumulation per unit of time (dM_T/dt) depends on the nitrogen uptake rate of the plant (dN_T/dt) and on the nitrogen utilisation efficiency (dM_T/dN_T) of the species (Hunt *et al.*, 1990). In the model, the nitrogen utilisation efficiency is a species-specific conversion parameter that describes the amount of biomass that a species can produce per unit weight of nitrogen taken up. Hence, plant growth is given by:

$$dM_T/dt = dM_T/dN_T \times dN_T/dt \quad (2)$$

The nitrogen utilisation efficiency (dM_T/dN_T) is assumed to remain constant during growth. We assume that differences in growth rate among the model species are exclusively caused by differences in nitrogen utilisation efficiency. Furthermore, we assume that (1) the relative allocation of biomass to leaves versus roots is equal among the model species, (2) that this allocation factor remains constant during plant growth, and (3) that this allocation factor is not influenced by the distribution of nitrogen over the patches. Let ξ be the allocation of biomass to the leaves relative to the roots and let β be the degree of selective allocation of root biomass to the nitrogen-rich patch, then:

$$dM_L/dt = \xi \times dM_T/dt \quad (3)$$

$$dM_{Rr}/dt = (1-\xi) \times \beta \times dM_T/dt \quad (4)$$

$$dM_{Rp}/dt = (1-\xi) \times (1-\beta) \times dM_T/dt \quad (5)$$

Nitrogen uptake and environmental heterogeneity

The nitrogen uptake rate of the plant depends on the amount of root biomass in the nutrient-rich and the nutrient-poor patch given by M_{Rr} and M_{Rp}, respectively, on the nitrogen absorption rate of the roots (Φ_n) and on the nitrogen concentration in those patches, respectively given by N_{Ar} and N_{Ap}. Hence, the nitrogen uptake rate can be described as:

$$dN_T/dt = (M_{Rr}\, \Phi_n\, N_{Ar} + M_{Rp}\, \Phi_n\, N_{Ap}) \quad (6)$$

The nitrogen uptake rate per unit root biomass is assumed to be non-saturating and to be a linear function of the local nitrogen concentration. In the model, nitrogen uptake does not result in depletion of the patches and as a result the nitrogen concentration in both patches remains constant.

Environmental heterogeneity is created by varying the nutrient concentration of the patches under the provision that the average nitrogen concentration (N_{AM}) over both patches is kept constant. Different heterogeneous environments are created based upon their patch contrast. Patch contrast (c) refers to the ratio of the nitrogen concentration in the nitrogen-rich (N_{Ar}) over that in the nitrogen-poor patch (N_{Ap}). Patch contrast (c) and the average nitrogen concentration (N_{AM}) are described as:

$$c = \frac{N_{Ar}}{N_{Ap}} \text{ and } N_{AM} = \frac{N_{Ar} + N_{Ap}}{2} \quad (7)$$

By maintaining a constant patch contrast in the model, the maximum effect of selective root placement on plant biomass accumulation is to be expected. Plants experience a homogeneous environment if patch contrast is 1. In the heterogeneous environments plants experience a patch contrast that is higher than 1.

Table 1. List of parameter units and values used in the model.

Symbol	Description	Unit	Starting condition
M_T	Plant biomass dry weight (DW)	g	0.30
M_L	Leaf biomass (DW)	g	0.18
M_{Rr}	Root biomass in rich patch (DW)	g	0.06
M_{Rp}	Root biomass in poor patch (DW)	g	0.06
dM_T/dN_T	Nitrogen utilisation efficiency	$g\ g^{-1}$	20-40
N_{AM}	Average nitrogen concentration of the soil solution	$g\ l^{-1}$	0.01
N_{Ar}	Nitrogen concentration of the soil solution in the nitrogen-rich patch	$g\ l^{-1}$	0.01-0.02
N_{Ap}	Nitrogen concentration of the soil solution in the nitrogen-poor patch	$g\ l^{-1}$	0.00-0.01
Φ_n	Soil solution absorption rate of the roots	$l\ g^{-1}\ d^{-1}$	1
ξ	Relative biomass allocation to the leaves	unitless	0.6
β	Relative biomass allocation to roots in rich patch	unitless	0.5-1
C	Patch contrast	unitless	1-∞
R	Relative growth rate	$g\ g^{-1}\ d^{-1}$	
H	Ratio of plant biomass in heterogeneous over that in homogeneous environments	unitless	

Analytical solution

In the model, the relative growth rate of a plant (r) is a function of the nitrogen utilisation efficiency of a species (dM_T/dN_T) and of the nitrogen uptake rate of the plant (dN_T/dt). From combining equations 2, 6 and 7 it follows that r is given by:

$$r = \frac{dM_T}{dN_T}(1-\xi)\Phi n N_{AM}\frac{c\beta + 1 - \beta}{1+c} \qquad (8)$$

The biomass produced by a plant (M_T) at time t is a function of the relative growth rate of the species (r), the initial biomass of the plant ($M_T(0)$), the length of the growth period and of the

initial distribution of plant dry weight over the three (i.e. M_L, M_{Rr} and M_{Rp}) plant compartments (I), and can be expressed as (see Appendix):

$$M_T(t) = \frac{1}{r}((I + rM_T(0))e^{rt} - I) \tag{9}$$

$$I = M_L(0) + M_{Rr}(0) + M_{Rp}(0) \tag{10}$$

The relative importance of morphological plasticity and relative growth rate for the biomass production of plants in heterogeneous environments can be assessed by comparing plant biomass accumulation in heterogeneous environments with that in homogeneous environments. The ratio of total plant biomass in the heterogeneous environment over that in the homogeneous environment (H) is given by:

$$H = \frac{M_T(t)_{(HETEROGENEOUS)}}{M_T(t)_{(HOMOGENEOUS)}} \tag{11}$$

Using the definitions of patch contrast (c), average nitrogen concentration (N_{AM}) and time (t) this equation yields:

$$H \approx \frac{\frac{1}{2}(1+c)}{(c\beta + 1 - \beta)} e^{2\frac{dMT}{dNT}(1-\xi)\Phi n N_{AM}\left(\frac{(c\beta+1-\beta)}{1+c} - \frac{1}{2}\right)t} \tag{12}$$

The analytical derivation of the model is given in detail in the Appendix. Selective root placement varies between non-selective root placement (β=0.5; i.e., plants place equal amount of roots in both patches) and fully selective root placement (β=1; i.e., plants place all roots in the nutrient-rich patch). Differences in relative growth rate are determined by differences in nitrogen utilisation efficiency. The effects of differences among plants in selective root placement and relative growth rate on the ratio of total plant biomass in the heterogeneous environment over that in the homogeneous environment can now be studied independently, both at a common point in time and at a common plant biomass. To assess the ratio at a common plant biomass, plants were compared when they reached a specific dry weight in the homogeneous environment.

Results

Not surprisingly, the ability to selectively place roots in nutrient-rich patches in heterogeneous environments (i.e. $\beta>0.5$) enhanced the biomass accumulation of plants. For a given patch contrast, plants accumulated relatively more biomass in the heterogeneous treatment compared to the homogeneous treatment with larger β (Fig. 1A, 1B). When comparisons were made at a common point in time (Fig. 1A), the effects of β were larger for plants with a higher inherent growth rate (i.e. higher nitrogen utilisation efficiency). Note that without selective root placement (i.e. $\beta=0.5$) a higher inherent relative growth rate did not result in a higher biomass accumulation of plants in heterogeneous environments relative to homogeneous environments (Fig. 1A). Strikingly, when comparisons were made at a common plant biomass instead of at a common point in time, the effects of selective root placement (β) did not differ among species with different inherent growth rates (Fig. 1B). The ratio of biomass accumulation in heterogeneous compared to homogeneous environments increased equally for all species with larger β and in cases of larger patch contrast (Fig. 1B).

For a given degree of selective root placement (β), relative plant biomass accumulation (H) increased with larger patch contrast (Fig. 2A, 2B). Note that if patch contrast is 1, plants experience a homogeneous environment. When compared at a common point in time, the ratio of plant biomass accumulation in heterogeneous over that in homogeneous environments at a specific patch contrast was larger at higher nitrogen utilisation efficiency (i.e. a higher relative growth rate) (Fig. 2A). When comparisons were made at a common plant biomass instead of at a common point in time, nitrogen utilisation efficiency did not affect the ratio of plant biomass accumulation in heterogeneous over that in homogeneous environments at a specific patch contrast (Fig. 2B).

For a given nitrogen utilisation efficiency (i.e. inherent relative growth rate), relative biomass accumulation (H) increased with larger β and patch contrast (Fig. 3). However, if plants were non-selective in their root placement ($\beta=0.5$), plant biomass accumulation in the heterogeneous environment was equal to the homogeneous environment (Fig. 3).

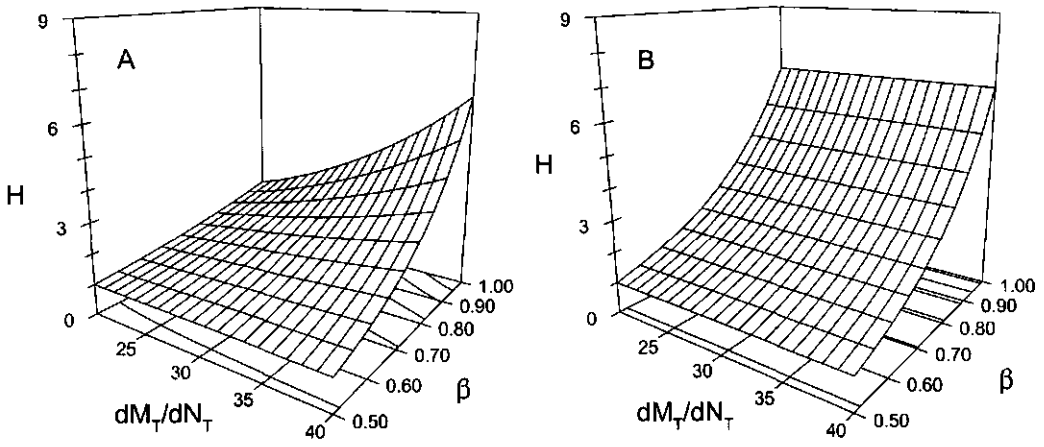

Figure 1. Ratio between plant biomass produced in heterogeneous environments and a homogeneous environment (H) as a function of selective root placement (β) and nitrogen utilisation efficiency (dM_T/dN_T) after a growth period of 28 days (A), and grown until the biomass of each plant in the homogeneous treatment is 30 g (B). Nitrogen utilisation efficiency is varied to create differences in plant relative growth rate. Furthermore if β=0.5 plants place their roots non-selectively and if β=1 plants place all roots in the richer patch. In all simulations, patch contrast (c), i.e. the ratio of the nitrogen concentration in the nitrogen-rich patch over that in the nitrogen-poor patch, is the same (c = 3). The average nitrogen concentration of the soil solution (N_{AM}) = 0.01 g l^{-1}.

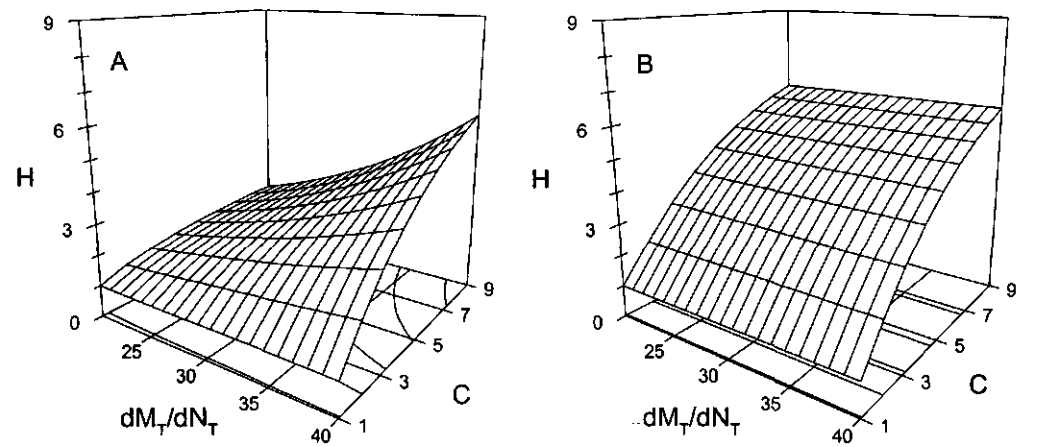

Figure 2. Ratio between plant biomass produced in heterogeneous environments and a homogeneous environment (H) as a function of patch contrast (c) and nitrogen utilisation efficiency (dM_T/dN_T) after a growth period of 28 days (A), and grown until the biomass of each plant in the homogeneous treatment is 30 g (B). Nitrogen utilisation efficiency is varied to create differences in plant relative growth rate. Patch contrast (c) is the ratio of the nitrogen concentration in the nitrogen-rich patch over that in the nitrogen-poor patch. Furthermore if β=0.5 plants place their roots non-selectively and if β=1 plants place all roots in the nitrogen-rich patch. In all simulations selective root placement (β) is the same (β = 0.8) and the average nitrogen concentration of the soil solution (N_{AM}) = 0.01 g l^{-1}.

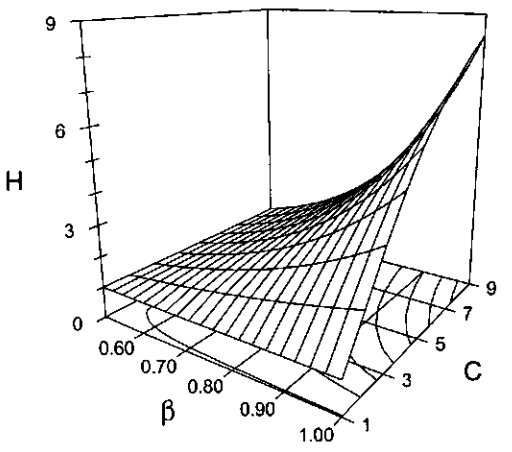

Figure 3. Ratio between plant biomass produced in heterogeneous environments and a homogeneous environment (H) as a function of patch contrast (c) and selective root placement (β). Patch contrast (c) is the ratio of the nitrogen concentration in the nitrogen-rich patch over that in the nitrogen-poor patch. Furthermore if β=0.5, plants place their roots non-selectively and if β=1 plants place all roots in the nitrogen-rich patch. In all simulations, nitrogen utilisation efficiency is the same (dM_T/dN_T = 30 g g^{-1}) and average nitrogen concentration (N_{AM}) = 0.01 g l^{-1}. In these simulations, H is the same when analysed at a common point in time or when analysed at a common weight.

Discussion

The present model was developed to disentangle the effects of root foraging and relative growth rate on the biomass accumulation of plants in heterogeneous environments. Root foraging is defined in the model as the selective allocation of root biomass per unit of time to the nitrogen-rich patch. Growth rate differences among the model plants result from differences in nitrogen utilisation efficiency. In the model, the degree of selective root placement can vary independently of plant growth rate.

Expected results were that the relative effects of selective root placement would be larger if roots were more selectively placed into the nitrogen-rich patch compared to the nitrogen-poor patch and if the patch contrast in the heterogeneous environment was higher. The model also demonstrated that selective root placement stimulates plant biomass accumulation in heterogeneous environments relative to homogeneous environments (Fig. 1) even though the average nitrogen concentration remains constant in all environments. This result is in accordance with empirical evidence (Birch and Hutchings, 1994; Fransen *et al.*, 1998).

A somewhat less straightforward model result is that without selective root placement (i.e. β=0.5), plants, irrespective of their inherent growth rate, do not accumulate more biomass in the heterogeneous environments than in the homogeneous environment (Fig. 1A, 1B). Without selective root placement, plants with a higher growth rate produce, in absolute terms,

more root biomass in the richer patch than plants with a lower growth rate, which should result in higher nitrogen acquisition and biomass accumulation by plants with higher growth rates. However, the higher acquisition is proportional to the higher growth rate of the plants. Consequently, high inherent growth rate per se does not confer an advantage in terms of biomass accumulation in heterogeneous compared to homogeneous environments.

On the other hand if plants are able to selectively place roots into the richer patch, a positive interaction exists between the degree of selectivity and growth rate on biomass accumulation in the heterogeneous environment. When compared at a common point in time, the relative effect of selective root placement on plant biomass accumulation in heterogeneous environments is larger when plants have a higher inherent growth rate (Fig. 1A). Hence, in terms of whole plant biomass accumulation selective root placement is more beneficial for fast-growing species than for slow-growing species. However, this is only true when plant biomass is compared at a common point in time. When plants are compared at a common biomass (Fig. 1B), selective root placement is equally beneficial for slow-growing species as for fast-growing species.

If comparisons made at a common point in time generate different results than those made at a common biomass, then what evaluation is most appropriate for comparing the foraging abilities between species?

Plants growing with different rates will be of different sizes when compared at a common point in time and may have different patterns of biomass partitioning over the various plant parts (Evans, 1972; Coleman *et al.*, 1994; Coleman and McConnaughay, 1995). However, if plants follow the same developmental trajectory, there may be no differences in biomass partitioning pattern when these plants are compared at equal sizes. Plasticity in traits representing any aspect of plant biomass should therefore be examined as a function of common biomass (Evans, 1972). Because foraging is accomplished by morphological plasticity, differences in foraging ability between species need to be examined at a common size instead of at a common time (see Hutchings and De Kroon, 1994). By doing so, our model suggests that relative growth rate does not enhance foraging ability on top of selective root placement.

In most empirical studies, morphological plasticity is typically analysed at a common point in time (Coleman *et al.*, 1994; but see Rice and Bazzaz, 1989 for a notable exception). The length of the growth period is identical for each species even though the inherent growth

rate of the species is different. Our model results show that faster-growing species will produce relatively more roots in the richer patches, and obtain relatively more nutrients from these patches, than slower-growing species when comparisons are made at a common point in time, even when their plasticity (i.e. their degree of selectivity) is the same. Hence, while empirical evidence suggests that faster-growing species are also more plastic than slower-growing species, this conclusion cannot be derived from the empirical studies carried out thus far because of the interaction between growth rate and plasticity. Future empirical studies that wish to assess the differences in plasticity between species need to evaluate plants at a common biomass.

It should be realised that the ecological advantages of growth rate and foraging ability in nature may well be evaluated after a given period of time rather than at a common weight (Coleman *et al.*, 1994). For example, when fast- and a slow-growing species compete for a finite, local nitrogen-rich patch, fast-growing species are able to generate a higher amount of root biomass in the nitrogen-rich patch after a given period of time than slow-growing species. Other things being equal, the species with the highest root biomass in the nitrogen-rich patch will capture most of the nitrogen from the patch (Nye and Tinker, 1977; Robinson *et al.*, 1999). The faster-growing species will acquire an even greater proportional share of the patchy resources when they are more plastic. Therefore, one may predict that high growth rate and high plasticity may have evolved concomitantly to enhance the capture of ephemeral patchy resources in a competitive environment. As explained above, the currently available comparative data cannot test this prediction because the effects of plasticity and growth rate on nutrient capture cannot be disentangled. In the model, we assume that plants grow exponentially, nitrogen concentration in the patches is constant, and plant parts do not senesce. We deliberately choose these conditions because, albeit unrealistic, under these assumptions, the largest possible effects of selective root placement and growth rate on plant biomass accumulation in heterogeneous environments as well as their maximum possible interactions are generated. However, these assumptions have important consequences for the model results. In reality, the effects of foraging and growth rate as well as their interactions will be less prominent than suggested by the model. For example, plants will only grow exponentially for a limited period of time and, hence, the ratio of plant biomass accumulation in heterogeneous over that in homogeneous habitats increases only linearly and not exponentially. Furthermore, increased nitrogen uptake in nitrogen-rich patches will normally

result in the depletion of the patch (Van Vuuren *et al.*, 1996). Ultimately, plants will not be able to accumulate more biomass in the heterogeneous than in the homogeneous environments if N exhaustion occurs, irrespective of their root foraging ability and growth rate (Fransen *et al.*, 1998; Hodge *et al.*, 1998). Finally, senescence of plant parts will reduce differences in biomass accumulation in different environments between plants that differ in growth rate, because species with a high growth rate will lose more biomass than species with a low growth rate (Aerts and Berendse, 1989; Vázquez de Aldana *et al.*, 1996).

In summary, when analysed at a common plant weight, selective root placement enhances plant biomass accumulation in heterogeneous compared to homogeneous environments, but growth rate does not. What now is the essential difference between foraging and growth? As noted before (Hutchings and De Kroon, 1994), foraging results from morphological responses and is concerned with the placement of resource acquiring structures within the heterogeneous surroundings of the plant whereas growth refers to the production of new biomass. While some morphological responses are simply expressions of growth, our model indicates that foraging distinguishes itself from growth by the selective (localised) occurrence of the response - enhanced proliferation of resource acquiring structures (such as roots) is expressed locally in the resource-rich patch only. This selectivity is a trait by itself that is critical for the foraging ability of the plant and is independent of growth rate.

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Appendix

For equation (3) we find:

$$\frac{dM_L}{dt} = \xi \frac{dM_T}{dt} \Leftrightarrow \int_0^t dM_L = \xi \int_0^t dM_T \Leftrightarrow M_L(t) - M_L(0) = \xi\left(M_T(t) - M_T(0)\right) \quad \text{(A1)}$$

Applying the same method for equation (4) and (5) results in:

$$M_{Rr}(t) - M_{Rr}(0) = (1-\xi)\beta\left(M_T(t) - M_T(0)\right) \quad \text{(A2)}$$

$$M_{Rp}(t) - M_{Rp}(0) = (1-\xi)(1-\beta)\left(M_T(t) - M_T(0)\right) \quad \text{(A3)}$$

Substitution of equation (6) in equation (2) results in:

$$\frac{dM_T}{dt} = \frac{dM_T}{dN_T}\left(M_{Rr}(t)\Phi_n N_{Ar} + M_{Rp}(t)\Phi_n N_{Ap}\right) \quad \text{(A4)}$$

Substitution of (A2) and (A3) gives after some rearrangement of the terms:

$$\frac{dM_T}{dt} = rM_T(t) + I \quad \text{(A5)}$$

with

$$r = \frac{dM_T}{dN_T}\left(\Phi_n N_{Ar}(1-\xi)\beta + \Phi_n N_{Ap}(1-\xi)(1-\beta)\right) \quad \text{(A6)}$$

$$I = -rM_T(0) + \frac{dM_T}{dN_T}\Phi_n N_{Ar} M_{Rr}(0) + \frac{dM_T}{dN_T}\Phi_n N_{Ap} M_{Rp}(0) \quad \text{(A7)}$$

In which r is the relative growth rate and I is determined by the initial distribution of plant biomass over the three plant compartments. I>0 because $M_{Rr}(0) \leq M_T(0)$, $M_{Rp}(0) \leq M_T(0)$ and $\xi \leq 1$, $\beta \leq 1$.

Using the method of separations of variables we find for equation (A5):

$$\begin{aligned} &\frac{dM_T}{dt} = rM_T(t) + I \Leftrightarrow \frac{1}{rM_T + I}\frac{dM_T}{dt} = 1 \Leftrightarrow \\ &\frac{1}{r}\ln(rM_T + I) = t + \tilde{C} \Leftrightarrow M_T(t) = \frac{1}{r}\left(e^{rt+r\tilde{C}} - I\right) \end{aligned} \quad \text{(A8)}$$

$\tilde{C}$ can be calculated because on t=0 applies $M_T(0)$:

$$M_T(0) = \frac{1}{r}\left(e^{r\tilde{C}} - I\right) \Rightarrow e^{r\tilde{C}} = rM_T(0) + I \quad \text{(A9)}$$

Substitution gives as solution of equation (A5):

$$MT(t) = \frac{1}{r}\left((I + rMT(0))e^{rt} - I\right) \tag{A10}$$

From this it follows that for $t \Rightarrow \infty$, $M_T(t)$ increases exponentially with growth rate *r*. Possible initial "deviations" from this exponential growth are caused by the initial conditions, i.e. the distribution of plant biomass over the three plant compartments (I). If the initial conditions deviate strongly from the final distribution of plant biomass over the plant compartment, the system first has to 'adapt' before the plant starts to grow exponentially.

Because M_T grows exponentially, it follows from (A1)-(A3) that all variables grow exponentially with growth rate r. From (A1)-(A3) we also see that eventually:

$$\frac{ML}{MT} = \xi; \frac{MRr}{MT} = (1-\xi)\beta; \frac{MRp}{MT} = (1-\xi)(1-\beta) \tag{A11}$$

The relative importance (H) of root selective placement and relative growth rate for biomass production in heterogeneous compared to homogeneous environments can be expressed as:

$$H = \frac{MT(t)_{(HETEROGENEOUS)}}{MT(t)_{(HOMOGENEOUS)}} \tag{A12}$$

In the following formulas are the parameters for the heterogeneous environment and the homogeneous environment given by the index (h) and (o) respectively.

$$H = \frac{\frac{1}{rh}\{Ih + rhMT(0))e^{rht} - Ih\}}{\frac{1}{ro}\{Io + roMT(0))e^{rot} - Io\}} \tag{A13}$$

for $t \Rightarrow \infty$:

$$H \approx \frac{\frac{1}{rh}\{Ih + rhMT(0))e^{rht}\}}{\frac{1}{ro}\{Io + roMT(0))e^{rot}\}} \tag{A14}$$

substituting equation (A7) and assuming $M_{Rr}(0) = M_{Rp}(0)$ gives after some further manipulations:

$$H \approx \frac{r_o}{r_h} e^{(r_h - r_o)t} \tag{A15}$$

Substituting equation (A5) finally yields:

$$H \approx \frac{\Phi nNAM}{\left(\Phi nNAr\beta + \Phi nNAp(1-\beta)\right)} e^{\frac{dMT}{dNT}(1-\xi)\left(\left(\Phi nNAr\beta + \Phi nNAp(1-\beta)\right) - \Phi nNAM\right)t} \tag{A16}$$

which using the definitions of patch contrast (c) and average nitrogen concentration (N_{AM}) yields:

$$H \approx \frac{\frac{1}{2}(1+c)}{(c\beta + 1 - \beta)} e^{2\frac{dMT}{dNT}(1-\xi)\Phi nNAM\left(\frac{(c\beta + 1 - \beta)}{1+c} - \frac{1}{2}\right)t} \tag{A17}$$

Chapter 3

Root morphological and physiological plasticity of perennial grass species and the exploitation of spatial and temporal heterogeneous nutrient patches

Bart Fransen, Jaap Blijjenberg and Hans de Kroon

Holcus lanatus L.

Plant and Soil, 1999 in press

Abstract

Root morphological and physiological characteristics of four perennial grass species were investigated in response to spatial and temporal heterogeneous nutrient patches. Two species from nutrient-rich habitats (i.e. *Holcus lanatus* and *Lolium perenne*) and two species from nutrient-poor habitats (i.e. *Festuca rubra* and *Anthoxanthum odoratum*) were included in the study. Patches were created by injecting equal amounts of nutrient solution into the soil either on one location (i.e. spatial heterogeneity) or on several, alternating locations (i.e. temporal heterogeneity) within the pot. The consequences of changes in root morphology and the implications for the exploitation of the nutrient patches by individual plants were quantified by the amount of ^{15}N captured from the enriched patches. The effects of nutrient heterogeneity on the acquisition of nutrients by species were determined by comparing the total nitrogen and phosphorus acquisition of the species in the two heterogeneous habitats with the total nitrogen and phosphorus acquisition in a homogeneous treatment. In this homogeneous treatment the same amount of nutrient solution was supplied homogeneously over the soil surface. The experiment lasted for 27 days and comprised one harvest. In response to the spatial enrichment treatment, all species produced significantly more root biomass within the enriched patch. The magnitude of the response was similar for species from nutrient-rich and nutrient-poor habitats. In contrast to this response of root biomass, root morphology, including specific root length, branching frequency and mean lateral root length was not affected by the treatments. In response to the temporal enrichment treatment, all species were able to increase the nitrogen uptake rate per unit of root biomass. The species from nutrient-poor habitats had, on average, higher uptake rates per unit root biomass than the species from nutrient-rich habitats, but the magnitude of the response did not differ between the species. These results question the general validity of the assumptions that root foraging characteristics differ among species from nutrient-rich and nutrient-poor habitats.

As a result of these root responses, all species captured an equal amount of ^{15}N from the spatial and temporal enriched nutrient patches and all species acquired significantly more nitrogen in the heterogeneous treatments than in homogeneous treatment. Hence, the ability to exploit local and temporal nutrient heterogeneity does not appear to differ between species from nutrient-rich and nutrient poor habitats, but is achieved by these species in different ways. The ecological implications of these differences are discussed.

Keywords: heterogeneity, morphological plasticity, ^{15}N-uptake, nutrients, physiological plasticity, roots

Introduction

Nutrients are heterogeneously distributed in natural habitats, both in space and time (Jackson and Caldwell, 1993; Gross *et al.*, 1995; Ryel *et al.*, 1996). Plants have developed foraging mechanisms that enable them to alter their root morphology and physiology in response to nutrient enrichment. It has been shown frequently that plants are able to proliferate roots as a result of morphological changes (Jackson and Caldwell, 1989; Gross *et al.*, 1993; Pregitzer *et al.*, 1993; Bilbrough and Caldwell, 1995; Larigauderie and Richards, 1994), and to increase their nutrient uptake rate per unit root biomass or length (Robinson and Rorison, 1983; Jackson *et al.*, 1990; Jackson and Caldwell, 1991; Robinson *et al.*, 1994; Van Vuuren *et al.*, 1996) as a result of physiological changes. However, both timing and degree of proliferation and the degree of physiological plasticity appears to be highly variable among species from different habitats.

Evidence is accumulating that inherently fast-growing species from nutrient-rich habitats display a higher degree of root morphological plasticity in response to nutrient enrichment than inherently slow-growing species from nutrient-poor habitats (Crick and Grime, 1987; Caldwell *et al.*, 1991; Campbell *et al.*, 1991; Fransen *et al.*, 1998; Robinson and Van Vuuren, 1998). Increased root proliferation results in increased nitrogen capture except if patch depletion occurs (Fransen *et al.*, 1998; Hodge *et al.*, 1998). Slow-growing species are assumed to maintain a large, long-lived root system which remains viable under prolonged periods of nutrient stress and that enables them to instantaneously increase the nutrient uptake capacity of roots in response to nutrient pulses (Grime *et al.*, 1986; Hutchings and de Kroon, 1994). However, evidence that species from nutrient-poor habitats display a higher degree of root physiological plasticity in response to nutrient enrichment than species from nutrient-rich habitats is still scarce (Robinson and Rorison, 1983; Campbell and Grime, 1989; Robinson and Van Vuuren, 1998).

Comparative studies that tested for differences in root foraging characteristics between species, including both morphological and physiological plasticity, have used plants of species that differed widely in relative growth rate, thereby including species of different families and growth-form (e.g. Crick and Grime, 1987; Campbell *et al.*, 1991). Differences in growth form and phylogeny between species of different plant families may thus have confounded the comparisons (Felsenstein, 1985; Harvey *et al.*, 1995). In addition, root foraging characteristics have been determined by comparing plants in heterogeneous environments with homogeneous environments that were either nutrient-rich or nutrient-poor. The larger relative differences shown by fast-growing species between these environments may have been due to their higher growth rate, rather than to their higher degree of root morphological plasticity. The differences in nutrient availability between the environments may have enabled fast-growing species to generate larger relative differences than slow-growing species (Fransen *et al.*, 1999). Hence, foraging characteristics may have been confounded with differences in growth form and growth rate. As a result root foraging differences between species of nutrient-rich vs. nutrient poor habitats, as well as the effects of root foraging differences, particularly of physiological plasticity, for the nutrient capture of species in heterogeneous environments are still poorly understood.

In this study we test the ability of four grass species, that occur along a gradient of soil nutrient availability, to exploit ephemeral nutrient patches. We use two grass species of nutrient-rich habitats and two grass species of nutrient-poor habitats. Four grass species are used to avoid confounding effects of gross differences in growth form and phylogeny between species of different plant families.

The species were subjected either to spatial patchiness, temporal patchiness or a homogeneous fertilisation treatment. The total amount of nutrients supplied to the plants was equal in all treatments. The application of ^{15}N-enriched nutrient solution enabled us to quantify the amount of nitrogen captured from the enriched patches and to estimate the nitrogen uptake rate per unit root biomass over the course of the experiment. To determine the consequences of nutrient heterogeneity for the acquisition of nutrients we measured the total nitrogen and phosphorus content of the plants and the plant biomass production. In this study we test the following hypotheses:

1) Species from nutrient-rich habitats display a higher local increment in root biomass in response to spatial nutrient enrichment than species from nutrient-poor habitats, and, as a result, species from nutrient-rich habitats are better able to capture nutrients from spatial nutrient patches than species from nutrient-poor habitats.
2) Species from nutrient-poor habitats display a higher local increase in nitrogen uptake rate in response to temporal nutrient enrichment than species from nutrient-rich habitats, and, as a result, species from nutrient-poor habitats are better able to capture nutrients from temporal nutrient patches than species from nutrient-poor habitats.

Materials and methods

Species

The four perennial grass species studied are characteristic of habitats which differ widely in nutrient availability within Western Europe. *Lolium perenne* L. and *Holcus lanatus* L. are fast-growing species (potential RGR=1.30 and 1.56 $week^{-1}$ respectively (Grime and Hunt, 1975)) characteristic species of nutrient-rich habitats. *Festuca rubra* L. and *Anthoxanthum odoratum* L. are species with intermediate growth rates (potential RGR=1.18 and 0.94 $week^{-1}$ respectively (Grime and Hunt, 1975)) characteristic of habitats that are moderately nutrient-poor.

The original plants of the four species were collected at different sites in a former agricultural grassland along the Anlooër Diepje, a brook in the 'Drentse Aa' Nature Reserve (53°N, 6°40'E) (see Bakker, 1989). The nutrient availability of the selected sites differed (Olff *et al.*, 1994) since the application of fertiliser to the sites was stopped in different years (Olff *et al.*, 1990). The plants used in this study are propagated from the field material in a heated greenhouse with supplemental lighting from high-pressure sodium lamps (Philips SON-T 400W) giving a photoperiod of 12h.

Materials

Young tillers, isolated from 4 original plants of each grass species were grown individually in 5-litre pots (17×17×18 cm) that were filled with a 5:1 mixture of coarse sand and humus-rich black soil. Soil nitrate-N, ammonium-N and phosphate-P (all extracted with 1M KCl) were 5.8 mg/kg, 3.0 mg/kg and 0.2 mg/kg, respectively. The pots were filled with a bulk soil density of 1.4 kg/dm^3. During the experiment plants were supplementary lighted for 16 h (400 W m^{-2}) with average temperatures of 20°C (day) and 15°C (night). Soil moisture was kept constant at 8% (mass %) by weighing and watering the pots with distilled water every 2 days beginning 4 weeks prior to the start of the enrichment treatments.

In each pot a window (diameter 7.4 cm) was cut in one side of the pot. The enrichment treatments started when roots were visible through the window of each of the pots. The window was cut 4 cm below the soil-surface and sealed with a transparency grid (5×5 mm). To minimise root exposure to light, each pot was placed inside a tight fitting, intact pot.

Enrichment treatments

The experiment consisted of a spatial and a temporal nutrient enrichment treatment and a homogeneous (control) treatment. Twelve pots (5 l) of each species were randomly assigned to each treatment. The pots were arranged in six blocks, each block containing two replicates of each species-treatment combination. Nutrient enrichment consisted of adding 15 ml of Steiner's universal nutrient solution (Steiner, 1961) without trace elements containing 0.2 mmol $Ca(NO_3)_2$, 0.125 mmol KNO_3, 0.022 mmol KH_2PO_4, 0.045 mmol $MgSO_4$ and 0.032 mmol K_2SO_4 every other day.

In both the spatial- and the temporal-enrichment treatment, the nutrients were added by injecting the nutrient solution with a syringe into the soil. Nutrients were injected at a depth of 5 cm. Each pot was divided into four imaginary quadrants, and in each quadrant the injection point was placed in the middle, 2 cm from the side of the pot. In the spatial enrichment treatment, nutrients were always injected in the same quadrant (Figure 1). In the temporal enrichment treatment, the nutrient injection point varied among the four imaginary quadrants in a pot, under the conditions that: 1) each quadrant received an equal number of

nutrient injections, and 2) on day 1, 9, and 17, the nutrients were injected in the quadrant next to the window side (i.e. quadrant 1; Figure 1). In the control treatment, the nutrient solution was spread homogeneously over the soil surface with a syringe every other day (Figure 1). On those days that no nutrients were added pots were weighed and watered with distilled water. The experiment that lasted for 27 days and consisted of 3 injection cycles (Figure 1) and 3 days.

To quantify the nitrogen uptake of the species from the enriched patches, plants were supplied on day 1, 9 and 17 with ^{15}N-enriched Steiner's universal nutrient solution. This solution was equal to the Steiner solution described above, but contained 7.0 μmol $K^{15}NO_3$ (99.3 atom% ^{15}N) and 0.118 mmol KNO_3, instead of 0.125 mmol KNO_3.

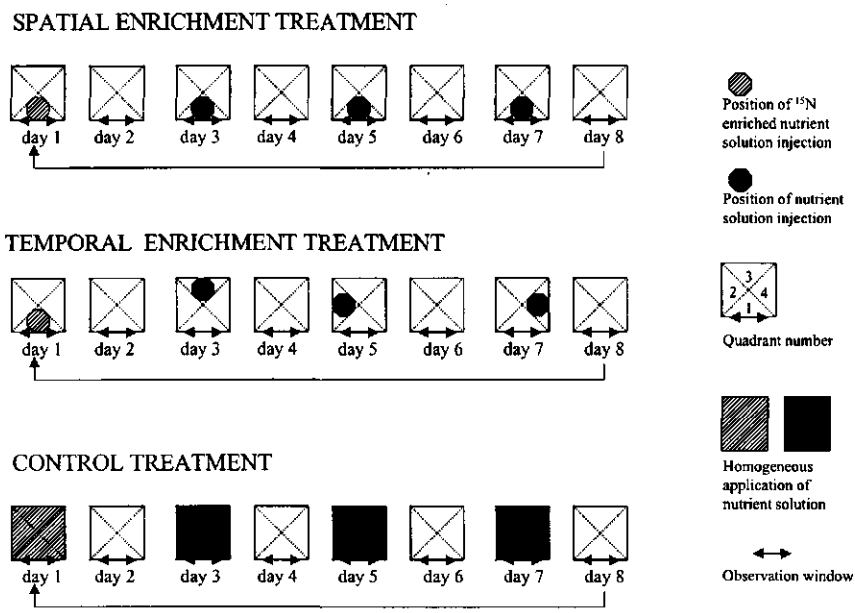

Figure 1. An 8-day nutrient injection cycle is shown for the spatial enrichment, temporal enrichment and homogeneous enrichment treatment. Each pot (5 l) was divided into four imaginary quadrants. In the spatial and temporal enrichment treatment, 15-ml Steiner nutrient solution was injected into the soil to a depth of 5 cm with a syringe. In the homogeneous treatment, 15-ml nutrient solution was spread over the soil surface. In the spatial enrichment treatment nutrient were injected on the same location. In the temporal enrichment treatment the injection points were varied temporally under the condition that each quadrant received an equal amount of nutrient injection. ^{15}N was added to the nutrient solution on set days. The 8-day nutrient injection cycle is repeated 3 times during the experiment.

Nutrient pulse size and duration

The actual size that an enriched patch would reach after the injection of 15-ml nutrient solution was determined in a pilot study. In this study, a nutrient solution containing nitrite was injected into soil that contained nitrite-indicator. It showed that the injection of 15-ml nutrient solution at a depth of 5 cm produces an enriched patch with a radius of 3 cm instantaneously.

To determine the persistence of the nutrient patches in the spatial- and temporal enrichment treatment, an additional experiment was carried out simultaneously with the main experiment. Four additional replicates of both the spatial and temporal enrichment treatments were grown with young plants of *Holcus lanatus*. In each of these pots a ceramic suction cup (height 8 cm, diameter 1.5 cm) was installed in the pots, 1 cm beneath the nutrient injection point. The ceramic suction cups enabled the extraction of soil moisture after the injection of nutrient solution. Soil moisture was extracted to quantify the changes in soil nutrient availability in the enriched patches after nutrient addition.

Measurements

At the start of the enrichment treatments (i.e. 4 weeks after the tillers were planted in the pots) 6 plants of each species were harvested, and shoot and root biomass was determined. At the harvest at end of the enrichment treatments, soil-cores (Ø 5.0 cm, depth 15 cm) were taken in each quadrant. Roots in these soil-cores were washed clean from soil particles. From the soil-cores taken in quadrant 1 in each treatment, three main adventitious roots of approximately 5 cm with all laterals were collected, and used for morphological observations. The rest of the root system was also washed clean from soil particles and collected.

The dry weight of the shoots, the roots in the soil-cores and the rest of the root system were determined after drying at 70°C for 48 h. The total N and P concentration in the shoots and the root system outside the soil-cores was measured using a continuous flow analyser (SKALAR, The Netherlands) after digesting the dried plant material with sulphuric acid, selenium, salicylic acid and perhydrol (Novozamsky *et al.*, 1983).

The ^{15}N concentration of the shoots was determined using an Isotope Ratio Mass Spectrometer (ANCA-IRMS). The absolute amount of ^{15}N in a plant (C) is obtained using the following equation:

$C=15 \times A \times B/(1400 + B)$

Here A = weight of the total N in the plant, and B = ^{15}N atom% in the shoots. Values of B are corrected by subtracting the natural abundance of ^{15}N in the plant material (0.366%). The ^{15}N atom% in the roots was equal to that in the shoots.

Following Drew and Saker (1975) the average ^{15}N-uptake rate per unit root biomass per day of the species in the spatial and temporal enrichment treatment was calculated as:

$d^{15}N/dt = ((C_2-C_1)/(t_2-t_1)) \times ((\ln(DW_2)-\ln(DW_1))/(DW_2-DW_1))$

Here C = the absolute amount of ^{15}N in the plant at the beginning (C_1) and the end (C_2) of the experiment, t = time, and DW = dry weight of the roots in the soil-core taken in quadrant 1 at the beginning (DW_1) and the end of the experiment (DW_2). The radius (2.5 cm) and depth (15 cm) of the soil-core taken coincide with the enriched soil column beneath the enriched patch.

The average ^{15}N-uptake rate per unit root biomass per day of the species in the control treatment could not be calculated, since the amount of root biomass that is involved in the uptake of ^{15}N could not be determined. The depth to which the homogeneously spread nutrient solution infiltrates into the soil is unknown and hence, the amount of root biomass involved in the uptake is unknown.

The soil moisture extracted in the additional experiment was analysed colorimetrically for NO_3^- and PO_4^- using a continuous-flow analyser (SKALAR, The Netherlands).

Statistical analyses

All data were analysed using analysis of variance (GLM-procedure; SPSS 1995) with species and treatment as spatial factors and block as a random factor. The data were checked for deviations from normality and for homogeneity of variances prior to analysis and transformed where necessary. A posteriori comparisons were carried out with Tukey's honest significant difference test where appropriate.

Results

Nutrient pulse duration

Differential nutrient application in the spatial- and temporal enrichment treatment resulted in a large temporal variation in both nitrate and phosphate availability between these two treatments. Spatial enrichment resulted in a series of short nitrate pulses; nitrate availability in the enriched patch fluctuated between consecutive days. Temporal enrichment resulted in a longer random nitrate pulse; the nitrate availability gradually declined and disappeared after 5 days within an 8 days enrichment series (Figure 2A). In contrast to nitrate, phosphate availability remained constant in the spatial enrichment treatment whereas in the temporal enrichment treatment, phosphate declined gradually within the 8 days period (Figure 2B).

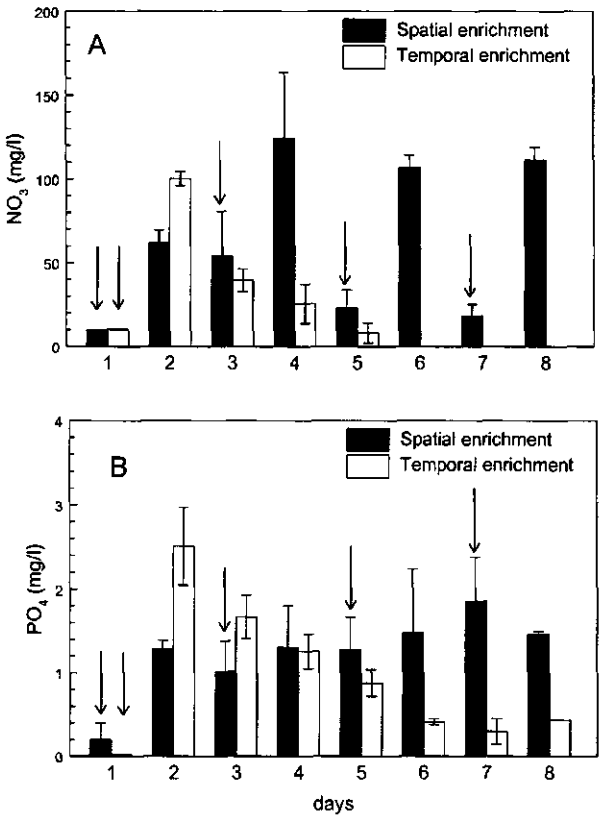

Figure 2. Concentration of nitrate (A) and phosphate (B) in the soil moisture extracted from quadrant 1 in the spatial and temporal enrichment treatment of the additional experiment. Arrows indicate the days on which 15 ml nutrient solution was injected into the quadrant. Data are means ± SE (n=4).

Root morphology

Plants did not alter their root morphology in response to the different enrichment treatments. Neither specific root length (SRL), nor branching frequency (i.e. the number of laterals per unit main axis) nor mean lateral root length of roots collected in quadrant 1 (see Figure 1) were significantly affected by treatment (Table 1).

Table 1. Analysis of variance using a general linear model for specific root length, branching frequency, mean lateral root length and root biomass of *Holcus lanatus* (Hl), *Lolium perenne* (Lp), *Festuca rubra* (Fr) and *Anthoxanthum odoratum* (Ao). Data are based on roots present in the soil-core taken in quadrant 1 of each treatment (see Figure 1). Data are means ± (SE), n=12.

	Specific root length ($m\ g^{-1}$)				Branching frequency (cm^{-1})			
	Hl	Lp	Fr	Ao	Hl	Lp	Fr	Ao
Spatial	154 ± (25)	243 ± (47)	105 ± (9)	244 ± (15)	6.0 ± (0.7)	4.5 ± (0.7)	4.0 ± (0.3)	6.5 ± (0.5)
Temporal	146 ± (14)	239 ± (48)	105 ± (12)	228 ± (11)	6.2 ± (0.6)	4.1 ± (0.5)	3.4 ± (0.6)	5.1 ± (0.4)
Control	133 ± (23)	158 ± (47)	105 ± (12)	208 ± (14)	6.4 ± (0.7)	4.0 ± (0.5)	3.9 ± (0.5)	5.5 ± (0.6)
Note	Species: P<0.001 Treatment: ns Spec × Treat: P<0.05				Species: P<0.001 Treatment: ns Spec × Treat: ns			

	Mean lateral root length (cm)				Root dry weight (mg)			
	Hl	Lp	Fr	Ao	Hl	Lp	Fr	Ao
Spatial	0.72 ± (0.13)	0.98 ± (0.12)	0.58 ± (0.07)	0.85 ± (0.14)	168 ± (12)	123 ± (19)	33 ± (4)	81 ± (7)
Temporal	0.63 ± (0.09)	0.95 ± (0.16)	0.84 ± (0.14)	0.74 ± (0.06)	82 ± (8)	58 ± (8)	23 ± (4)	36 ± (5)
Control	0.66 ± (0.08)	0.89 ± (0.23)	0.64 ± (0.11)	0.82 ± (0.09)	72 ± (10)	67 ± (14)	15 ± (3)	31 ± (5)
Note	Species: ns Treatment: ns Spec × Treat: ns				Species: P<0.001 Treatment: P<0.001 Spec × Treat: ns			

The significant species × treatment interaction for specific root length is caused by the lack of response of *Festuca rubra* to the different enrichment treatments. In all other species the specific root length declined gradually in the order spatial to temporal enrichment to control treatment, but the specific root length of *Festuca rubra* was equal in all treatments.

In contrast to root morphology, the amount of root biomass produced in quadrant 1 was significantly affected by treatment (Table 1). In the spatial enrichment treatment, in which quadrant 1 was constantly enriched, root biomass in this quadrant was significantly ($P<0.05$) higher than in the same quadrant in the temporal enrichment and the control treatment. The faster-growing species from nutrient-rich habitats (i.e. *Holcus lanatus* and *Lolium perenne*) produced on average significantly more root biomass in quadrant 1 than species from nutrient-poor habitats (i.e. *Festuca rubra* and *Anthoxanthum odoratum;* $F_{1,46}=38.87$, $P<0.001$; $F_{1,46}=30.67$, $P<0.001$ and $F_{1,46}=27.28$, $P<0.001$ for the spatial, temporal and control treatment respectively). However, the degree in which the root biomass in the spatial enrichment treatment is increased relative to the control treatment did not differ between the nutrient-rich and nutrient-poor species ($F_{1,45}=0.24$, $P=0.629$). *Holcus lanatus, Lolium perenne, Festuca rubra* and *Anthoxanthum odoratum* showed a 2.3 ± 0.36, 1.8 ± 0.48, 2.2 ± 0.52 and 2.6 ± 0.48 (mean ± SE) fold increase in root biomass in the spatial enrichment treatment relative to the control treatment respectively. Within the spatial enrichment treatment, the root biomass in quadrant 1 differed significantly from the root biomass within the three other quadrants taken within this treatment, except for *Festuca rubra* (Figure 3A). Since the roots in this treatment were morphologically similar to the roots in the temporal and control treatment (Table 1), the increased root biomass in quadrant 1 must be the result of a local increment of the number of main roots. Within the temporal enrichment (Figure 3B) and in the control treatment (Figure 3C) no differences in root biomass production were found between the four different quadrants.

^{15}N-uptake

On average the plants acquired 68% of the ^{15}N supplied, but the total amount of ^{15}N (Figure 4A) acquired differed significantly between the species and between the treatments (Table 2). Overall, the species acquired significantly ($P<0.05$) more ^{15}N in the spatial- and temporal

enrichment treatments than in the control treatment. When compared within species *Holcus lanatus* acquired significantly ($p<0.05$) more ^{15}N in the spatial- and temporal enrichment treatment than in the control treatment and *Anthoxanthum odoratum* acquired significantly ($P<0.05$) more ^{15}N in the temporal enrichment treatment than in the control treatment. For the other two species (i.e. *Lolium perenne* and *Festuca rubra*) the amounts of ^{15}N acquired were not significantly different between treatments.

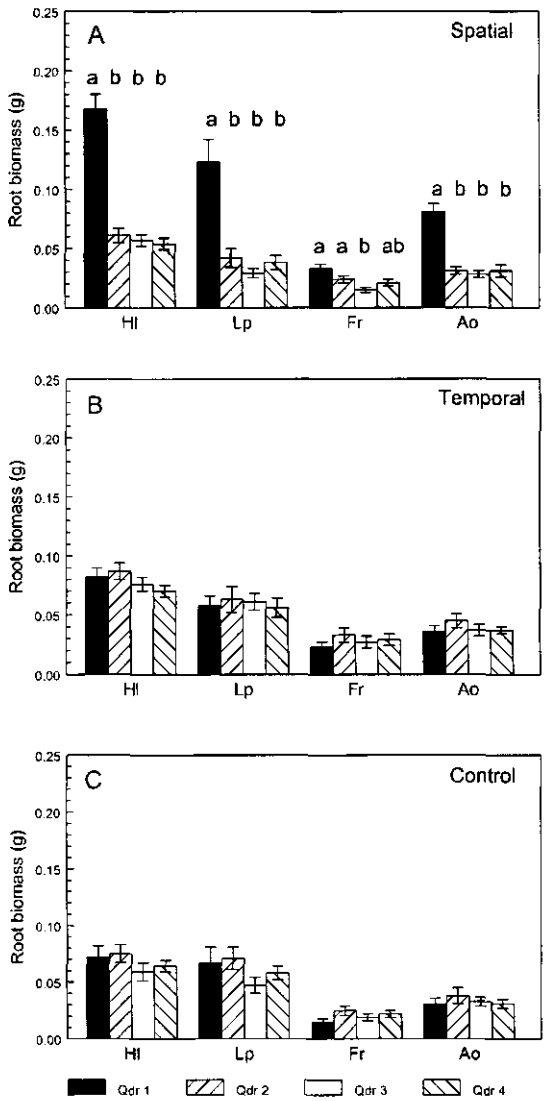

Figure 3. Root biomass in the soil-cores taken in the spatial (A), temporal (B) and control (C) treatment for *Holcus lanatus* (Hl), *Lolium perenne* (Lp), *Festuca rubra* (Fr) and *Anthoxanthum odoratum* (Ao). Data are means ± SE (n=12). Bars with the same letter within species are not significantly different (Tukey HSD test; $P>0.05$).

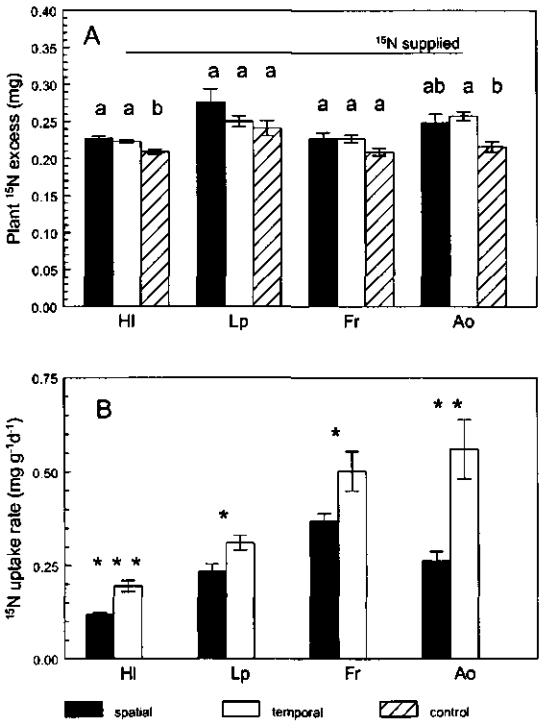

Figure 4. Total content of ^{15}N (A) in the spatial, temporal and control enrichment treatment, and ^{15}N-uptake rate uptake rate per unit root biomass in the spatial and temporal enrichment treatment averaged over the experiment (B) for *Holcus lanatus* (Hl), *Lolium perenne* (Lp), *Festuca rubra* (Fr) and *Anthoxanthum odoratum* (Ao). Data are means ± SE (n=6). Bars with the same letter within species are not significantly different (Tukey HSD test; P>0.05) (A). *Asterisks* indicate significant differences within species (B). *P<0.05. **P<0.01 and ***P<0.001.

Table 2 Analysis of variance using a general linear model for the total amount of ^{15}N-acquired (^{15}N), the ^{15}N-uptake rate per unit root biomass (NUR), total plant nitrogen content (total N), total plant phosphorus content (total P), and shoot, root and total plant biomass. F values are given.

Source	df	^{15}N	df	NUR	df	total N	total P	shoot	root	plant
Species (S)	3	12.48^{**}	3	40.56^{***}	3	7.89^{**}	28.82^{***}	36.42^{***}	48.00^{***}	42.18^{***}
Treatment (T)	2	24.57^{**}	1	167.63^{**}	2	13.75^{**}	1.31^{NS}	3.76^{NS}	0.05^{NS}	1.42^{NS}
Block (B)	2	3.90^{NS}	2	—[1]	5	1.84^{NS}	1.66^{NS}	3.04^{*}	3.34^{*}	2.75^{NS}
S * T	6	1.89^{NS}	3	1.47^{NS}	6	1.16^{NS}	1.19^{NS}	0.56^{NS}	0.41^{NS}	0.51^{NS}
S * B	6	1.56^{NS}	6	0.68^{NS}	15	7.01^{***}	5.37^{***}	5.41^{***}	2.63^{*}	4.62^{***}
T * B	4	0.67^{NS}	2	0.16^{NS}	10	0.63^{NS}	1.29^{NS}	0.81^{NS}	0.90^{NS}	0.91^{NS}
S * T * B	12	0.61^{NS}	24	2.64^{*}	30	0.79^{NS}	0.60^{NS}	0.42^{NS}	0.72^{NS}	0.50^{NS}

NS = not significant, * P<0.05, ** P<0.01, *** P<0.001

[1] Error degrees of freedom could not be calculated.

By combining the amount of ^{15}N acquired by the species and the amount of root biomass produced in the ^{15}N enriched quadrant (i.e. quadrant 1), the average ^{15}N-uptake rate per unit root biomass can be calculated. The ^{15}N-uptake rates differed significantly between species and treatments (Table 2). All species had higher ^{15}N-uptake rates per unit root biomass in the temporal treatment than in the spatial treatment (Figure 4B). On average, the degree to which the ^{15}N-uptake rate increased did not differ ($F_{1,22}$=1.430, P=0.245) between the nutrient-rich species and nutrient-poor species. *Holcus lanatus, Lolium perenne, Festuca rubra* and *Anthoxanthum odoratum* increased their ^{15}N-uptake per unit root biomass per day respectively 1.7 ± 0.17, 1.4 ± 0.11, 1.4 ± 0.17 and 2.2 ± 0.28 (mean ± SE) fold. However, noteworthy is that the uptake rates of the nutrient-poor species (i.e. *Festuca rubra* and *Anthoxanthum odoratum*) were significantly higher than the uptake rates of the two nutrient-rich species (i.e. *Holcus lanatus* and *Lolium perenne*; ($F_{1,22}$=21.22, P<0.001 and $F_{1,22}$=37.27, P<0.001 for respectively the spatial and temporal treatment). Thus, the smaller amount of root biomass produced by these species in both the spatial and temporal enrichment treatments is compensated for by a higher nutrient uptake rate per unit root biomass.

Total plant N, P and biomass

The total plant nitrogen content (Figure 5A) showed a similar pattern as the ^{15}N-acquisition pattern. Overall, the species acquired significantly more nitrogen in the spatial- and temporal enrichment treatment than in the control treatment (Table 2), but when compared within species most of these differences were not significant. The total plant phosphorus content (Figure 5B) showed no significant difference between the different treatments.

The amount of total plant biomass (Figure 5C) produced during the experiment differed significantly between the species but was not affected by treatment. The faster-growing species *H. lanatus* and *L. perenne* produced significantly more total plant biomass than the slower-growing species *F. rubra* and *A. odoratum.* Hence, the overall differences in nitrogen acquisition between the treatments did not result in detectable biomass differences.

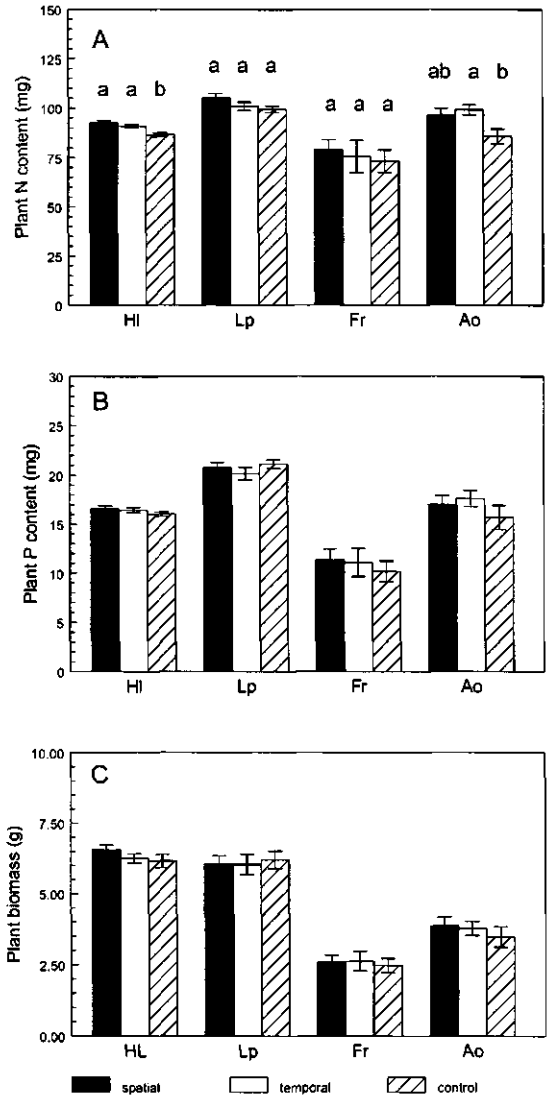

Figure 5. Total content of nitrogen (A), phosphorus (B) and total plant biomass (C) per plant for *Holcus lanatus* (Hl), *Lolium perenne* (Lp), *Festuca rubra* (Fr) *and Anthoxanthum odoratum* (Ao) in the spatial, temporal and control treatment. Data are means ± SE (n=12). Bars with the same letter within species are not significantly different (Tukey HSD test; P>0.05).

Discussion

The current experiment is among the first that compares the root foraging characteristics of several plant species from one family (Gramineae) in response to spatial and temporal nutrient

heterogeneity (Fransen *et al.*, 1998; Hodge, 1998). At the end of the 27 days experiment, no significant differences were detected in the degree of root morphological and physiological plasticity in response to nutrient heterogeneity of species from nutrient-rich and poor habitats even though large differences exist among the species in characteristics such as inherent relative growth rate. Hence, the experiment fails to demonstrate that root foraging characteristics differ among dominant species from habitats of different successional stages or nutrient availability (Grime *et al.*, 1986; Grime, 1994; Hutchings and De Kroon, 1994) and the concomitant differential ability of the species to exploit ephemeral nutrient pulses.

The species from nutrient-rich habitats (i.e *Holcus lanatus and Lolium perenne*) produced on average significantly more root biomass in the spatial and temporal enrichment patches than the species from nutrient-poor habitats (i.e. *Festuca rubra* and *Anthoxanthum odoratum*). However, all species showed a similar increase in root biomass in response to the spatial nutrient enrichment when compared to the homogeneous control treatment. Surprisingly, root morphology (i.e. specific root length, branching frequency and mean lateral root length) of the species was not affected by the different enrichment treatments. Hence, we conclude that the increased root biomass in the enriched patch in the spatial enrichment treatment must have resulted from a spatial increment in the number of main root axes. It is noteworthy, that the increment in root biomass in the enriched patch in the spatial enrichment treatment did not affect root growth in other parts of the root system. As observed by Fransen et al. (1998), the root biomass in the other three soil-cores taken in the spatial enrichment treatment was equal to the root biomass in the soil-cores taken in the temporal and control treatment.

The lack of differences in root morphological responses between the species in this study contrasts with the general idea that fast-growing species display a higher degree of root morphological plasticity than slow-growing species. For example, Robinson and Van Vuuren (1998) showed in their review that fast-growing species display on average a higher degree of root morphological plasticity than slow-growing species, when root morphologies in nutrient-rich patches and uniformly nutrient-deficient controls are compared. However, such differences between fast- and slow-growing species may be the result of growth rate differences (Fransen *et al.*, 1999). Differences in nutrient availability between the two environments may enable fast-growing species to generate larger relative differences in size than slow-growing species simply due to their higher relative growth rate, rather than from

differences in root morphological plasticity. In the current experiment, the overall nutrient availability in the different enrichment treatments was equal and this may explain that the species of different growth rate exhibited a similar degree of selective root placement. All species were able to increase their ^{15}N-uptake rate per unit root biomass in the temporal enrichment treatment relative to the spatial enrichment treatment. The degree of root physiological plasticity did not significantly differ between the species from nutrient-rich and nutrient-poor habitats. The increase in ^{15}N-uptake is exclusively due to physiological plasticity (i.e. elevated uptake kinetics) and does not result only from a higher inflow due to a higher nitrogen concentration (Caldwell *et al.*, 1992; Jackson and Caldwell, 1996). The nitrogen concentration in the ^{15}N enriched patch in the temporal treatment was, on average, even lower during the experiment than in the ^{15}N enriched patch in the spatial treatment. On average, species from nutrient-poor habitats had higher ^{15}N-uptake rate per unit root biomass than species from nutrient-rich habitats. Our data do not allow to tell whether these difference reflect actual species differences or are merely the result of the smaller amount of root biomass produced by the former species.

The total nitrogen content of a plant differed significantly between species and treatment (Table 2). All species, irrespective of the nutrient availability of their natural habitat or their inherent relative growth rate, captured more nitrogen and phosphorus in the heterogeneous treatments than in the homogeneous treatment. Hence, by the end of a 27 days experiment, species from nutrient-rich and nutrient-poor habitats are equally able to exploit ephemeral nutrient patches. Other studies have also shown that when equal amounts of nutrients are distributed heterogeneously instead of homogeneously, plants can acquire more nitrogen (Fransen *et al.*, 1998) and produce more biomass (Birch and Hutchings, 1994). The importance of root plasticity for the acquisition of nutrients in heterogeneous environments is shown when these empirical results are compared to the modelling results of Jackson and Caldwell (1996) and Ryel and Caldwell (1998). These latter studies showed that model plants that do not display root plasticity acquire less, instead of more, nutrients in heterogeneous than in homogeneous environments, even though the total nutrient availability is invariant.

In conclusion, our results provide no indications that the root foraging characteristics differ between grass species from habitats that differ in nutrient availability. All species showed similar degrees of root morphological and physiological changes in response to nutrient heterogeneity. As a result, all species acquired similar amounts of ^{15}N from the

enriched nutrient patches in the spatial and temporal enrichment treatment, but the species from nutrient-rich habitats achieved this in a different way than the species from nutrient-poor habitats. The two species from nutrient-rich habitats produced more root biomass than species from nutrient-poor habitats, while the two species from nutrient-poor habitats had higher ^{15}N-uptake rates per unit root biomass than species from nutrient-rich habitats. Hence, perennial species from nutrient-rich habitats seem indeed dependent on high root biomass production to sustain high nutrient capture rates in heterogeneous environments, as is suggested by several authors (Grime *et al.*, 1986; Sibly and Grime 1986; Crick and Grime 1987). They hypothesised that species from nutrient-rich habitats depend on high root production to adjust biomass partitioning within the root system to sustain high nutrient capture rates in heterogeneous environments. However, the high root turnover rates of species from nutrient-rich patches are disadvantageous in nutrient-poor habitats (Grime *et al.*, 1986; Sibly and Grime, 1986; Berendse *et al.*, 1987; Berendse and Elberse, 1990; Berendse, 1994). Hence, the more persistent root system and the possibly high nutrient uptake rates per unit root biomass as generally displayed by species from nutrient-poor habitats seems beneficial in their natural habitat (Grime *et al.*, 1986, Sibly and Grime, 1986; Crick and Grime 1987).

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Chapter 4

Root morphological plasticity and nutrient acquisition of perennial grass species from habitats of different nutrient availability

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Festuca rubra L.

Oecologia 115: 351-358, 1998

Abstract

We studied the root foraging ability and its consequences for the nutrient acquisition of five grass species that differ in relative growth rate (RGR) and that occur in habitats that differ widely in nutrient availability. Foraging responses were quantified, based on the performance of the plants in homogeneous and heterogeneous soil environments of the same overall nutrient availability. Although all species tended to produce a significantly higher root length density in a nutrient-rich patch, this response was significant only for the faster-growing species. The increased root length density resulted from small, though not significant, changes in root biomass and specific root length.

The effectiveness of root proliferation was determined by quantifying the total amount of nutrients (N and P) accumulated by the plants over the course of the experiment. Plant acquired more N in a heterogeneous environment than in a homogeneous environment, although the total nutrient availability was the same. The ability to acquire nutrients (N or P) in the heterogeneous environment was not related to the ability of species to increase root length density in response to local nutrient enrichment.

In contrast to other studies, our results suggest that the role of morphological plasticity of roots to acquire patchily distributed resources is limited. Possible reasons for this discrepancy are discussed.

Keywords: foraging, morphological plasticity, nutrient heterogeneity, perennial grasses, root proliferation

Introduction

Spatial and temporal resource heterogeneity is ubiquitous in natural ecosystems (Caldwell and Pearcy, 1994). Nutrients are patchily distributed in the soil at scales relevant to individual plants (Jackson and Caldwell, 1993a,b; Kotliar and Wiens, 1990; Stuefer, 1996). Plants have developed foraging mechanisms that enable them to acquire adequate amounts of resources in these heterogeneous environments (see Hutchings and De Kroon, 1994 and references therein). Foraging in plants is accomplished by morphological changes in response to environmental conditions, and may result in the selective placement of resource acquiring structures (leaves and roots) within the environment (Grime *et al.*, 1986; Hutchings and Slade, 1988; Hutchings and De Kroon, 1994; De Kroon and Hutchings, 1995).

Several studies have shown the ability of plants to proliferate roots in nutrient-rich patches, i.e. to produce high root length density in nutrient-rich patches (Drew, 1975; Crick and Grime, 1987; Jackson and Caldwell, 1989; Gross *et al.*, 1993; Pregitzer *et al.*, 1993; Larigauderie and Richards, 1994; Bilbrough and Caldwell, 1995). The degree of root proliferation is nutrient specific (Drew, 1975; Jackson and Caldwell, 1989), modulated by soil nutrient concentration (Jackson and Caldwell, 1989) and plant nutrient demand (Caldwell, 1994), but is also species specific (Crick and Grime, 1987; Jackson and Caldwell, 1989; Caldwell *et al.*, 1991; Robinson, 1994).

To explain this latter variation, it has been hypothesised that root foraging characteristics differ among species from habitats of different successional stage or nutrient status (Grime *et al.*, 1986; Grime, 1994; Fitter, 1994; Hutchings and De Kroon, 1994). Root foraging characteristics of fast-growing species from nutrient-rich habitats will be characterised by high levels of morphological plasticity which allows an extension from the localised nutrient depletion zones that are a consequence of the high nutrient uptake achieved by these plants (Grime, 1994).

In contrast, slow-growing species from nutrient-poor habitats are assumed to depend on a large long-lived root system which remains viable under prolonged conditions of nutrient depletion (Grime, 1994; Hutchings and De Kroon, 1994). Especially the ability to reduce nutrient losses is an important feature determining the success of species in nutrient-poor habitats (Berendse, 1994; Berendse and Elberse, 1990). Slow-growing species are therefore assumed to respond to environmental heterogeneity primarily by physiological plasticity (Grime, 1994: Hutchings and De Kroon, 1994). Physiological plasticity is the enhancement of

the nutrient uptake capacity per unit root length in response to localised soil enrichment (Jackson *et al.*, 1990; Jackson and Caldwell, 1991; Robinson, 1994; Van Vuuren *et al.*, 1996). These differential responses to nutrient heterogeneity between fast- and slow-growing species may explain the documented changes in species composition during succession in natural plant communities.

In this study we test this hypothesis by examining the root foraging abilities of a range of grass species that differ in relative growth rate (RGR), and that occur along a gradient of soil nutrient availability. By using species of a single plant family, confounding effects of gross differences in growth form and phylogeny (Felsenstein, 1985; Harvey *et al.*, 1995) are avoided. Root morphological changes in response to nutrient heterogeneity are assessed as local root biomass production and as changes in specific root length (SRL, root length per unit root dry weight). Root proliferation in nutrient-rich patches is measured as root length density (RLD, root length per unit soil volume). The consequences of root foraging for the nutrient acquisition of plants (total N and P taken up) in spatially heterogeneous environments are also determined. We hypothesise that:

1) Faster-growing species from more nutrient-rich habitats will increase root biomass and specific root length in nutrient-rich patches, resulting in a higher root length density in nutrient-rich compared to nutrient-poor patches.
2) Nutrient acquisition in spatially heterogeneous environments is positively correlated with the ability to generate a high root length density in the nutrient-rich patches.

Materials and methods

Plant species

This experiment was carried out with *Lolium perenne* L., *Holcus lanatus* L., *Festuca rubra* L., *Anthoxanthum odoratum* L. and *Nardus stricta* L., all common perennial grasses with a wide distribution in Western Europe (Weeda, 1994). *Lolium perenne* L. and *Holcus lanatus* L. are fast-growing species, *Festuca rubra* L. and *Anthoxanthum odoratum* L. are species with an

intermediate relative growth rate (RGR) and *Nardus stricta* L. is a slow-growing species (Grime and Hunt, 1975).

The plant material used in the experiment originated from fields along the Anlooër Diepje, a brook in the 'Drentsche Aa' Nature Reserve (53°N,6°40'E). The management in these former agricultural grasslands changed from cutting twice a year and fertilising (100-200 kg N ha^{-1} $year^{-1}$) to cutting once a year (July) without fertilisation (Bakker, 1989). Fertiliser application was stopped at different years (Olff *et al.*, 1990). Hence, fields in which the management changed only recently are still relatively nutrient-rich whereas fields in which the management changed a long time ago are nutrient-poor (Olff *et al.*, 1994). Fields with different management duration represent different stages in a reversed successional gradient (Olff *et al.*, 1994). As a consequence of the decline in nutrient availability during this reversed succession the species composition of the fields changed. The pasture species *Lolium perenne* L. is replaced by *Holcus lanatus* L. shortly after fertilisation stops, and *Holcus lanatus* L. is gradually replaced by subsequently *Festuca rubra* L., *Anthoxanthum odoratum* L. and *Nardus stricta* L. (Olff and Bakker, 1991; Olff *et al.*, 1990).

The original plants (i.e. a group of tillers) of each species were collected from several fields along the Anlooër Diepje in March 1995 and cloned in a common garden in Wageningen. Plants taken from different fields, are genotypically different. In August 1995 young tillers of four genotypes of each species were isolated from the garden plants and grown individually in the greenhouse to ensure homogeneous start conditions. At the start of the experiment in September 1995, tillers were randomly assigned to either an initial harvest or to the experiment. In the experiment, each genotype was used in both treatments.

Experimental treatments and growing conditions

The experiment consisted of a homogeneous treatment and a heterogeneous treatment which were replicated 4 times. In both treatments the plants were grown individually in root-boxes (22 dm^3; Mechalectron B.V., The Netherlands). The perforated walls of the root-boxes were in this experiment covered with black plastic. The root-boxes were divided in half by a water tight PVC-partitioning that was sealed to the bottom and the walls of the root-box with plasticine (Rhiwa-Hartomex B.V., The Netherlands).

In the homogeneous treatment, both halves of the root-box were filled with a homogeneous mixture of humus-rich black soil and sand (ratio 1:3.5 v/v), hereafter referred to as nutrient-poor soil. The root-box was filled to a depth of 40 cm with a bulk soil density of 1.4 kg/dm^3.

In the heterogeneous treatment the same total amount of humus-rich black soil and sand were used. One half of the root-box in this treatment was filled with the same nutrient-poor soil that was used in the homogeneous treatment. In the other half a nutrient-rich patch was created by filling a central PVC-cylinder (diameter 7 cm, depth 25 cm) with a soil mixture in which the ratio humus-rich black soil:sand was 4:1 (v/v), hereafter referred to as nutrient-rich soil. The remaining part of this half of the root-box was filled with the remaining humus-rich black soil and sand in a ratio 1:5 (v/v). After filling, the PVC-cylinder was removed, and hence, no barrier existed between the nutrient-rich soil in the patch and the surrounding nutrient-poor soil. Roots could therefore freely penetrate the nutrient-rich patch. Both the nutrient-rich patch as well as the rest of the half were filled with the same bulk density of 1.4 kg/dm^3 as the nutrient-poor soil. In this way 30% of the humus-rich black soil was concentrated in 10% of the volume of this half of the root-box. The total amounts of humus-rich black soil and sand used in the experiment was equal in both halves and in both treatments.

At the start of the experiment, plants were placed on top of the divider separating both halves of the root-box. Around each plant a buffer-zone (nutrient-poor soil: 1:3.5 v/v humus-rich black soil, sand) was created to ensure equal soil conditions at both sides of the plant in order to enable the plant to equally distribute its roots over both halves of the root-box (Fig. 1). To diminish evaporation the soil surface was covered with a 1 cm thick layer of white plastic grains.

The experiment was carried out in a greenhouse. During the experiment (September-December) the plants were supplemented with light from high-pressure sodium lamps (Philips SON-T Plus 400 W) giving a photoperiod of 16 hours. Temperature was kept constant at 20°C (day) and 15 °C (night) and relative air humidity was kept at 70%. The plants were watered three times a week and the soil water content was monitored regularly with a Frequency Domain probe (IMAG-DLO, The Netherlands) and kept constant.

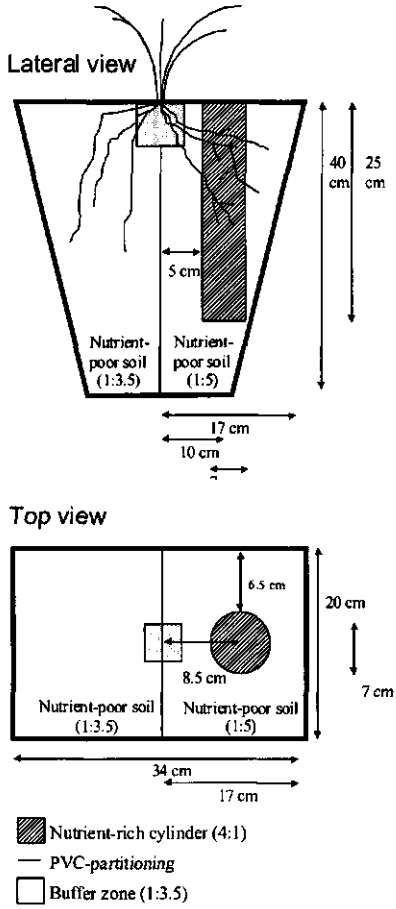

Figure 1. Experimental set-up of the root-boxes in the heterogeneous treatment. The root-boxes (22 dm^3) were divided in half by a water tight PVC partitioning. One half was filled with a nutrient-poor soil mixture of humus-rich black soil and sand (ratio 1:3.5 v/v). In the other half a nutrient-rich patch was created by filling a central patch with a humus-rich black soil-sand mixture (ratio 4:1 (v/v). The rest of this half was filled with the remaining humus-rich black soil and sand in a 1:5 (v/v) ratio. The roots could freely grow into the nutrient-rich soil in the patch; there was no barrier. The root-boxes in the homogeneous treatment were also divided by a PVC partitioning, but both halves were filled with the 1:3.5 nutrient-poor soil mixture. The total amount of humus-rich black soil and sand used in the experiment was equal in both halves and in both treatments. In both treatments a buffer zone (4x4x4 cm; nutrient-poor soil 1:3.5 v/v humus-rich black soil:sand ratio) was created around each plant to ensure identical conditions at the start of the experiment.

Harvest

In the initial harvest 10 plants per genotype of each species were harvested, and shoot and root biomass were determined. At the final harvest shoots were removed from the plants and soil-cores (diameter 5 cm, depth 0-10 cm and 10-20 cm) were taken in both treatments in both halves of the root-boxes. The centre of all soil-cores taken was equivalent to the position of

the centre of the nutrient-rich patch in the heterogeneous treatment, i.e. 8.5 cm from the shoot base (Fig. 1). The soil-cores were collected and the roots in these soil-cores were washed clean of soil particles, frozen and used later for the measurement of the root morphological parameters. The rest of the root system in either half of the root-box was washed clean at harvest, removed from the shoot-base, frozen and stored.

Comparisons among species have generally been conducted at common points in time or at a common plant age, but plants growing in different environments are likely to grow at different rates and will be of different sizes and developmental stage at a given time or age (Coleman *et al.*, 1994; Coleman and McConnaughay, 1995). Apparent differences in morphology may then be the result of ontogenetic drift rather than expressions of an adaptation to environmental heterogeneity (Coleman *et al.*, 1994). For this reason, the grass species were harvested sequentially to reduce size differences among species, and to enable a functional interpretation of the differences in foraging mechanisms. Following the rank of their relative growth rates, *Holcus lanatus*, *Lolium perenne*, *Festuca rubra*, *Anthoxanthum odoratum* and *Nardus stricta* were harvested after 8, 9, 10, 11 and 12 weeks, respectively.

Measurements

The nutrient concentrations and the pH of the initial soil mixtures and of the soil at the end of the experiment were determined by extracting 20 g of fresh soil with 50 ml KCl (1 M) for 2 hours. Soil pH was determined within the extract. Simultaneously, the soil water content was determined after drying soil at 105°C for at least 24 hours. The extracts were analysed colorimetrically for NH_4^+, NO_3^- and PO_4^- with a continuous flow analyser (SKALAR, The Netherlands). The total available soil nitrogen and phosphorus contents of the root-boxes in the homogeneous and heterogeneous treatments were calculated.

To determine the root morphological parameters, defrosted roots from the soil cores were stained by submerging them in 50 mg/l methylene blue for at least 12 hours, after which they were spread out in water trays and scanned with a 3D-scanner (TRUVEL TZ-3, Vidar System Corporation, Herndon, USA). Roots were stained to increase contrast and excessive dye was rinsed off with water before scanning. The total length of a root sample was determined by analysing the scanned images with the interactive image analysis package TCL-Image V4.6 (TNO Institute of applied Physics, Delft, The Netherlands). For details of

the procedure see Smit *et al.* (1994). After scanning, the dry weight of the root sample was determined and the specific root length (SRL) and the root length density (RLD) were calculated.

Leaves, shoot-base and the remaining roots of both the initial harvest and the final harvest were dried at 70°C for at least 48 hours prior to weighing and nutrient analyses. The dried plant material was digested with sulphuric acid, selenium and salicylic acid (Novozamsky *et al.*, 1983). Nitrogen and phosphorus concentrations were measured colorimetrically using a continuous flow analyser (SKALAR, The Netherlands).

Statistical analysis

The initial soil characteristics were analysed with one-way ANOVA. The total amount of available nutrients in the root-boxes in the homogeneous and the heterogeneous treatments were analysed with the Student's *t*-test. The experiment had a split-plot design (Sokal and Rohlf, 1981). The root morphological parameters, (i.e. root biomass production, specific root length and root length density) were analysed using a mixed-model ANOVA (Genstat5, Version 3.1), with species and treatments randomised over the root-boxes (4 replicates, 30 d.f. for error term) with the following block structure: half (nested within root-box, 2 halves, 30 d.f. for error term) and depth (nested within half, 2 depths, 59 d.f. for error term). Genotypic effects could not be considered in the analyses, since the genotypes were nested within species and the number of degrees of freedom was insufficient to allow for a nested analysis of genotype. Prior to analyses the root morphological data were $Y'=Y^{1/3}$ transformed, satisfying best the conditions of normality and homogeneity of variance. Total plant nutrient content, plant biomass and plant relative growth rate (RGR) were analysed with using a two-factor ANOVA with five species and two treatments (homogeneous, heterogeneous). Prior to analyses nutrient- and biomass data were ln-transformed to satisfy the conditions of normality and homogeneity of variance. RGR data were not transformed prior to analysis. Plant relative growth rate is calculated per genotype as:

$RGR = [\ln(dw_2)-\ln(dw_1)]/t$

where dw_2 is the final plant dry weight; dw_1 is the initial plant dry weight and t is the length of the growth period of the plant. The initial plant dry weight is the mean dry weight (n=10) of the plants harvested per genotype in the initial harvest.

Results

Soil characteristics

The initial ammonium, nitrate and phosphate concentrations of the soil in the nutrient-rich patch in the heterogeneous treatment differed significantly ($P<0.05$) from the concentrations of the nutrient-poor soil (Table 1). The total amount of ammonium ($t_s = 2.5$), nitrate ($t_s = 1.24$) and phosphate ($t_s = 1.45$) available in the root-boxes did not differ ($t_{0.05[2]} = 4.303$) between the treatments.

During the experiment, the ammonium and nitrate concentrations in the nutrient-rich soil declined significantly. The average soil water content during the experiment was 5.7 ± 0.1% (n=250) in the nutrient-poor (1:3.5) soil mixtures and 10.1 ± 0.2 % (n=105) in the nutrient-rich (4:1) soil mixture, corresponding with soil water potentials of -0.01 MPa and -0.02 MPa, respectively.

Table 1. Soil characteristics of the nutrient-poor (1:3.5) and nutrient-rich (4:1) humus-rich black soil and sand mixtures at the beginning and at the end of the experiment. Means with the same superscript letter are not significantly different (Least Significant Difference test (P=0.05) after one-way ANOVA). DW = dry weigth.

	Nutrient-poor soil		Nutrient-rich soil	
	initial (n=3)	end (n=24)	Initial (n=3)	end (n=8)
pH	4.9^{a}	5.18^{b}	4.6^{c}	4.72^{c}
NH_4^+ (mg N kg^{-1} DW)	0.15^{a}	0.27^{a1}	1.25^{b}	0.59^{a}
NO_3^- (mg N kg^{-1} DW)	8.20^{a}	0.60^{b2}	48.05^{c}	2.16^{b}
PO_4^- (mg P kg^{-1} DW)	0.22^{a}	0.20^{a}	0.54^{b}	0.48^{b}

[1]Denominator =17; [2]Denominator =12

Root responses

Overall, the species produced more root biomass in the soil-cores in the heterogeneous treatment than in the soil-cores in the homogeneous treatment ($P<0.01$; Table 2). However, when compared within species, no differences could be shown ($P>0.05$; Fig. 2A) between the root biomass production within the different soil-cores of both treatments.

Table 2. Analysis of variance of the effect of species, treatment, half and depth on root biomass allocation, specific root length (SRL) and root length density (RLD). The experiment was analysed as a split-plot design with species and treatment randomised over root-boxes, half nested within root-box and depth nested within half. Data were $Y'=Y^{1/3}$ transformed prior to analyses (x=57 for DW, 56 for SRL, 59 for RLD).

		F-values for each dependent variable		
Effect	d.f.	DW	SRL	RLD
Species	4,30	23.75 ***	43.27 ***	99.17 ***
Treatment	1,30	8.05 **	0.59^{NS}	10.72 **
Species x Treatment	4,30	0.13^{NS}	0.72^{NS}	0.77^{NS}
Half	1,30[a]	0.43^{NS}	13.17 ***	36.26 ***
Species x Half	4,30[a]	0.19^{NS}	0.61^{NS}	2.51^{NS}
Treatment x Half	1,30[a]	2.61^{NS}	12.14 **	61.31 ***
Species x Treatment x Half	4,30[a]	0.41^{NS}	3.04 *	4.32 **
Depth	1,x	52.32 ***	3.36^{NS}	98.66 ***
Species x Depth	4,x	18.65 ***	12.25 ***	7.60 ***
Treatment x Depth	1,x	0.19^{NS}	0.00^{NS}	0.75^{NS}
Half x Depth	1,x	0.00^{NS}	0.03^{NS}	0.09^{NS}
Species x Treatment x Depth	4,x	1.71^{NS}	3.00 *	4.16 *
Species x Half x Depth	4,x	0.27^{NS}	0.14^{NS}	0.42^{NS}
Treatment x Half x Depth	1,x	0.41^{NS}	0.03^{NS}	1.79^{NS}
Species x Treatment x Half x Depth	4,x	0.27^{NS}	0.44^{NS}	0.42^{NS}

[a]Denominator =29 for SRL *P<0.05, **P<0.01, ***P<0.001; NS not significant

Treatment did not affect the specific root length (SRL) of the species (Table 2). However, the SRL of *L. perenne* and *N. stricta* was significantly higher (P<0.05) in the nutrient-rich patch in the heterogeneous treatment than in the nutrient-poor half of that treatment. The SRL in the nutrient-rich patch did however not differ from the SRL in the nutrient-poor halves in the homogeneous treatment (Fig. 2B).

The concentration of nutrients in the nutrient-rich patch in the heterogeneous treatment significantly affected the root length density of the species (Table 2). All species (Table 2) tended to increase their root length density (RLD) in response to nutrient-enrichment. However, only *L. perenne, H. lanatus* and *F. rubra* were able to produce a significantly higher (P<0.05) RLD in the nutrient-rich patch in the heterogeneous treatment compared to both the

nutrient-poor half in that treatment and the nutrient-poor halves in the homogeneous treatment. Note, the RLD in the nutrient-poor half of the heterogeneous treatment was equal to the RLD in the nutrient-poor halves in the homogeneous treatment and hence, was independent of the nutrient availability experienced by the plant in the nutrient-rich patch (Fig. 2C). So the difference in RLD between the nutrient-rich patch and the nutrient-poor half in the heterogeneous treatment as shown by *L. perenne, H. lanatus* and *F. rubra* is the result of selective root placement within the nutrient-rich patch.

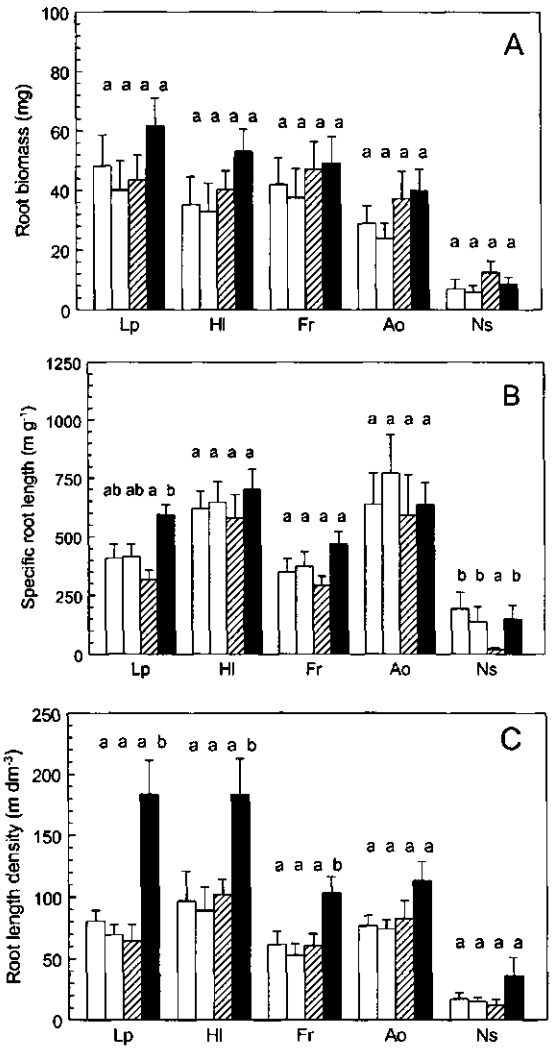

Figure 2. Root morphological parameters, including root dry weight (A), specific root length (B), and root length density (C) of *Lolium perenne* (Lp), *Holcus lanatus* (Hl), *Festuca rubra* (Fr), *Anthoxanthum odoratum* (Ao) and *Nardus stricta* (Ns).. Data are means ± SE (n=8) from the roots sampled from soil-cores taken within both nutrient-poor sides of the homogeneous treatment (open bars), the nutrient-poor side in the heterogeneous treatment (hatched bars), and within the nutrient-rich patch in the heterogeneous treatment (closed bars). Bars with the same letter within species are not significantly different (LSD-test; P>0.05). Note that *Holcus lanatus*, *Lolium perenne*, *Festuca rubra*, *Anthoxanthum odoratum* and *Nardus stricta* were harvested after 8, 9, 10, 11 and 12 weeks, respectively. The actual root length density of *Nardus stricta* (Ns) is multiplied by 10 in the figure.

Rooting characteristics also differed with soil depth (see Table 2; results not shown). In general, root biomass production decreased and specific root length increased with soil depth, except for *N. stricta* which showed exactly the opposite pattern. Nevertheless, all species showed a decrease in root length density with increasing soil depth.

Nutrient acquisition

The total amount of nitrogen acquired per plant (Fig. 3A) differed significantly between the species ($F_{4,30}$=15.11; P<0.001) and was affected by treatment ($F_{1,30}$=8.78; P<0.01). Overall, the plants in the heterogeneous treatment acquired more nitrogen than the plants in the homogeneous treatment even though the total amount of available nitrogen was equal. However, least significant difference (LSD) tests revealed that *A. odoratum* was the only species that acquired significantly (P<0.05) more nitrogen in the heterogeneous treatment than in the homogeneous treatment. The total amount of phosphorus acquired by the plants (Fig. 3B) differed significantly between the species ($F_{4,30}$=54.40; P<0.001), but was not affected by treatment ($F_{1,30}$=3.59; P>0.05).

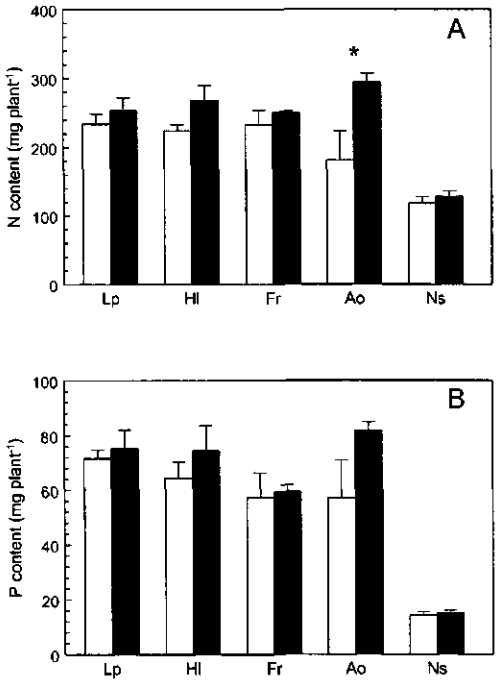

Figure 3. Total content per plant of (A) nitrogen and (B) phosphorus per plant of *Lolium perenne* (Lp), *Holcus lanatus* (Hl), *Festuca rubra* (Fr), *Anthoxanthum odoratum* (Ao) and *Nardus stricta* (Ns) in the homogeneous treatment (blank bars) and heterogeneous treatment (filled bars). Data are means ± SE (n=4). An asterisk indicates a significant difference (LSD-test; P<0.05) within species. Note: *Holcus lanatus*, *Lolium perenne*, *Festuca rubra*, *Anthoxanthum odoratum* and *Nardus stricta* were harvested after 8, 9, 10, 11 and 12 weeks, respectively.

Biomass production

Treatment affected total plant biomass production although the total amount of available nutrients was equal in both treatments. The species produced overall more plant biomass in the heterogeneous treatment (Table 3), but in a posteriori tests only *A. odoratum* showed a significant higher ($P<0.05$) plant biomass production in the heterogeneous treatment (Fig. 4A). The difference in total plant biomass between the two treatments was mainly due to the higher above-ground biomass production in the heterogeneous treatment (Table 3; Fig 4B); root biomass of the species was not affected by treatment (Table 3; Fig. 4C). The relative growth rate (RGR) of the species in the experiment differed significantly ($F_{4,30}=63.59$; $P<0.001$) and coincides with their ranking within the successional sequence in the field (Fig. 4D), except for *L. perenne*.

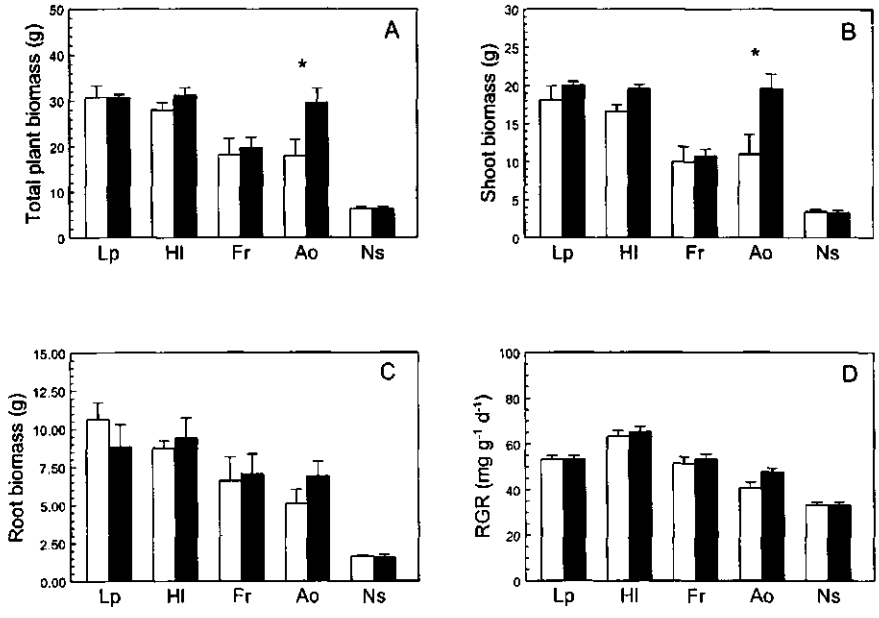

Figure 4. Plant **A** total biomass, **B** shoot biomass, **C** root biomass and **D** relative growth rate in the homogeneous treatment (blank bars) and the heterogeneous treatment (filled bars) for *Lolium perenne* (Lp), *Holcus lanatus* (Hl), *Festuca rubra* (Fr), *Anthoxanthum odoratum* (Ao) and *Nardus stricta* (Ns). Data are means ± SE (n=4). An asterisk indicates a significant difference (LSD-test; $P<0.05$) within species. Note: *Holcus lanatus*, *Lolium perenne*, *Festuca rubra*, *Anthoxanthum odoratum* and *Nardus stricta* were harvested after 8, 9, 10, 11 and 12 weeks, respectively.

Table 3 Analysis of variance of shoot biomass, root biomass and total plant biomass. Data are means ± SE (n=4). Data were ln-transformed prior to analysis.

		F values for each dependent variable		
Effect	d.f.	Leaves	Roots	Plant
Species	4,30	58.83 ***	38.17 ***	55.48 ***
Treatment	1,30	6.40 *	0.19 ^{NS}	4.35 *
Species x Treatment	4,30	2.15 ^{NS}	0.79 ^{NS}	1.70 ^{NS}

F-values are given with their level of significance: P*<0.05, **P<0.01, ***P<0.001; ns not significant

Discussion

Only the faster-growing species, i.e. *Lolium perenne, Holcus lanatus* and *Festuca rubra*, produced significantly higher root length densities in the nutrient-rich patch than in the nutrient-poor soil, although all species showed a quantitatively similar response. The increases in root length density were the result of small (insignificant) increases in root biomass production and specific root length (SRL) to nutrient enrichment. Remarkably, the acquisition of both nitrogen and phosphorus in the heterogeneous treatment was not related to the root proliferation ability of the species.

Root proliferation can be generated by a local increase in root biomass and/or specific root length. In contrast to our study, numerous experiments have shown that species are able to produce significantly more root biomass in nutrient-rich patches (see Robinson, 1994 for review). The discrepancy with these studies may be caused by the depletion of the nutrient-rich patch in our experiment. The nitrogen availability in the nutrient-rich patch declined drastically during the experiment (Table 1). In other studies (Drew, 1975; Drew and Saker, 1975; Drew and Saker, 1978; Crick and Grime, 1987; Granato and Raper, 1989; Campbell *et al.*, 1991) the nutrient concentrations applied to the roots were more constant as a result of continuous replenishment. The response shown by the species in our study might reflect a more realistic response of species to nutrient-rich patches in natural habitats. Species may initially allocate more root biomass to such enriched micro-sites, but when depletion of the local soil environment occurs root biomass production in the patch may stall. Such a flexible biomass allocation mechanism will enable species to reduce the risk of biomass and resource losses when patches become unprofitable.

Generally, enhanced root growth in nutrient-enriched patches may occur at the expense of root growth elsewhere in the root system (Gersani and Sachs, 1992; Fitter, 1994; Hutchings and De Kroon, 1994; Robinson, 1994). However, in our experiment the increased root length density of *Lolium perenne*, *Holcus lanatus* and *Festuca rubra* in the nutrient-rich patch did not compromise the root length density in the nutrient-poor half of the root-box. Robinson (1994) showed that root growth in nutrient-poor areas is only loosely correlated with root growth in nutrient-rich areas. Correlative growth among different parts within a root system will predominantly occur in young plants that do not have nutrient storage pools. These plants have to use the acquired nutrients immediately for growth and maintenance, and stimulated root growth in a nutrient-rich area results inevitably in a growth reduction in other parts in the root system. Large perennial plants with considerable amounts of accumulated nutrients are probably able to maintain growth in both nutrient-rich and nutrient-poor areas due to the ability to remobilize stored nutrients.

The significantly enhanced root proliferation in the nutrient-rich patch, as shown by *Lolium perenne*, *Holcus lanatus* and *Festuca rubra*, did not result in a significantly enhanced acquisition of nitrogen and phosphorus in the heterogeneous treatment. The only species that acquired significantly more nitrogen in the heterogeneous treatment, i.e. *A. odoratum*, did not produce significantly more roots in the nutrient-rich patch than in the nutrient-poor soil. There are reasons to assume that the increased nitrogen acquisition by *A. odoratum* is the result of physiological plasticity. Stimulated nutrient inflow rate in roots in nutrient-rich areas can result from increased uptake kinetics in response to nutrient enrichment, but can also simply result from the higher nutrient concentration in the soil-solution in these areas (Caldwell *et al.*, 1992; Jackson and Caldwell, 1996). However, if the latter was true in our study, all species would have shown increased nutrient acquisition in the heterogeneous treatment.

Our results suggest that the ecological significance of root morphological plasticity for the acquisition of heterogeneously distributed nutrients is limited. Also in other studies, physiological plasticity appears to be more important for the acquisition of nutrients than morphological plasticity (Caldwell *et al.*, 1992, Jackson and Caldwell, 1996; Robinson, 1996; Van Vuuren *et al.*, 1996). However, physiological plasticity may only be more beneficial when plants are grown individually or when soil water content allows high nutrient diffusion rates. When plants are grown in competition or when ions are immobile, the ability to rapidly

reach and fill nutrient-rich patches may be more significant for nutrient capture (cf. Robinson, 1994).

In conclusion our results show that, although all species tended to respond, only faster-growing species were able to produce significantly higher root length densities in the nutrient-rich patch. These increased root length densities were due to small, insignificant, increases in root biomass and specific root length. In contrast to our expectations, the response of root length density did not correlated with an increased nutrient acquisition in the heterogeneous treatment. For the one species for which the nitrogen acquisition was particularly high, these benefits were probably due to physiological plasticity.

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Chapter 5

Root proliferation, root turnover rates and biomass production of two perennial grass species from habitats of contrasting nutrient availability: the long-term disadvantages of selective root placement.

Bart Fransen and Hans de Kroon

Anthoxanthum odoratum L.

Abstract

In spite of the numerous root foraging studies, the long-term benefits of selective root proliferation in heterogeneous environments are still unknown. The duration of most foraging studies has been too short to include patch depletion and root turnover, but exactly these two effects may limit the rewards of root proliferation for perennial plants in natural habitats. Depletion, i.e. the gradual decline in the nutrient availability due to nutrient uptake and leaching, results in a lower nutrient uptake per unit root length or biomass, and root turnover results in nutrient losses. Here we describe the results of a long-term experiment in which the effects of both patch depletion and root turnover on the potential rewards of root proliferation are examined.

In our experiment, *Holcus lanatus* a perennial grass species characteristic of inherently nutrient-rich habitats and *Nardus stricta*, a perennial grass species characteristic of inherently nutrient-poor environments were grown in homogeneous nutrient-rich and homogeneous nutrient-poor treatments, and in heterogeneous treatments, under two levels of overall nutrient availability during two years. The patch contrast between the nutrient-rich and the nutrient-poor patch in the heterogeneous treatment was the same under both overall levels of nutrient availability. The two species have shown to differ in root foraging ability and were assumed to differ in root turnover rate. *H. lanatus* displays a high degree of selective root placement and is assumed to have a high root turnover rate. In contrast, *N. stricta* displays a more rigid pattern of root development and is assumed to have a low root turnover rate.

The species were expected to behave differently under the two overall levels of nutrient availability. In heterogeneous nutrient-rich habitats, *H. lanatus* may acquire sufficient nutrients, due to its high degree of selective root placement, to offset its high nutrient losses. However, in heterogeneous soils in overall nutrient-poor habitats, the gains in terms of nutrient uptake of selective root placement may be smaller, and hence, *H. lanatus* may not be able to acquire sufficient nutrients to offset its nutrient losses. In these habitats a more stable pattern and a lower root turnover rate, as displayed by *N. stricta*, may be more beneficial.

Surprisingly, *H. lanatus* produced relatively less shoot biomass than expected under both overall levels of nutrient availability, even though it produced significantly more root biomass in the nutrient-rich side of the heterogeneous treatment under the overall high level of

nutrient availability. In contrast, the shoot biomass production of *N. stricta* in the heterogenous treatment did not differ from the expected biomass based on the two homogeneous treatments in none of the two overall levels of nutrient availability.

Root longevity of the species, as determined by minirhizotron observations, revealed that roots of *H. lanatus* tended to live shorter during the experiment than roots of *N. stricta*. The lower than expected biomass production of *H. lanatus* in the heterogeneous treatments could, however, not be explained by diffences in root turnover between treatments. The root longevity of *H. lanatus* did not differ between the heterogeneous and homogeneous treatments at none of the two overall levels of nutrient availability. Presumably, *H. lanatus* produced more roots in the nutrient-rich side in the heterogeneous treatment than necessary to acquire the available nutrients. The increased root biomass in the nutrient-rich side in the heterogeneous treatment occurred without affecting root biomass in the nutrient-poor side in this treatment, hence, *H. lanatus* must have allocated more biomass to roots, inevitably resulting in a lower shoot biomass. The implications of these results for the long-term rewards of root proliferation for perennial species in heterogeneous environments are discussed.

Keywords: foraging, minirhizotrons, nutrient heterogeneity, morphological plasticity, root longevity.

Introduction

Natural habitats are intrinsically heterogeneous, both in space and time. Plants have developed foraging mechanisms, including root morphological and physiological alterations, in response to nutrient heterogeneity. These root foraging mechanisms are essential for plants to acquire adequate amounts of nutrients in heterogeneous habitats. Jackson and Caldwell (1996) and Ryel and Caldwell (1998) have shown theoretically that plants that do not alter root morphology or physiology in response to nutrient enrichment acquire less nutrients in heterogeneous environments than in homogeneous environments even though the total amount of nutrients available in both environments is equal.

In spite of the numerous foraging studies on root morphological plasticity, the long-term benefits of root proliferation are still unknown. There are at least two reasons to assume that the rewards of root proliferation for perennial plants in natural habitats may be lower than expected based on foraging studies carried out so far.

Firstly, in many root foraging studies the nutrient concentration in the enriched patches was kept constant during the experiment, but in natural habitats patch depletion occurs: a gradual decline in the nutrient supply of patches due to nutrient uptake and leaching. Patch depletion limits the potential benefits, in terms of nitrogen acquisition, of root proliferation in heterogeneous environments (Van Vuuren *et al.*, 1996; Fransen *et al.*, 1998). Strikingly, root proliferation may even occur after the plant has taken up most of the nitrogen available in the enriched patch (Van Vuuren *et al.*, 1996). Hence, root physiological plasticity may be more important for rapid nitrogen uptake prior to patch depletion than root proliferation (Van Vuuren *et al.*, 1996; Ryel and Caldwell 1998).

The effects of patch depletion are still poorly studied. The effects of patch depletion are likely to be important especially on the longer term, but most root foraging studies are typically carried out over a very short time-span, i.e. in the order of weeks. Our previous study on the root foraging ability of five perennial grass species (Fransen et al. 1998), was one of the longest carried out so far, but lasted only for 3 months.

Secondly, until now the duration of all root foraging studies has been too short to include effects of differences in root turnover rates between species. The merits of root foraging in response to nutrient heterogeneity are often defined in terms of nutrient uptake or short-term growth, but the long-term growth of perennial plants is dependent on the balance between nutrient uptake and nutrient losses due to turnover of plant parts (Berendse 1985; Berendse 1994a,b). In general, species from nutrient-rich habitats display a higher degree of root proliferation than species from nutrient-poor habitats (cf. Robinson and Van Vuuren, 1998), but they also lose more nutrients than species from nutrient-poor habitats, due to higher root turnover rates, leaching or exudation (Vázquez de Aldana *et al.*, 1996). Species from nutrient-rich habitats that display a high degree of root proliferation in enriched patches are able to acquire sufficient nutrients to offset the high nutrient losses if the overall level of nutrient supply is large enough. However, in nutrient-poor habitats, these species may not be able to acquire sufficient nutrients to offset their nutrient losses (cf. Berendse and Elberse, 1990), resulting in a disadvantage compared to species from nutrient-poor habitats that

display less root proliferation, but that are better able to retain captured nutrients due to longer tissue lifespan (Berendse *et al.*, 1987; Aerts, 1989; Diemer *et al.*, 1992).

In this study we examined the long-term benefits of root foraging for mature perennial grass species. The experiment lasted for two growing seasons and is, to our knowledge, the first foraging experiment that lasted long enough to include possible effects of differences in root turnover on foraging benefits. In the experiment, plants were grown in homogenous nutrient-rich and nutrient-poor environments, and in heterogeneous environments under field conditions in the Wageningen Rhizolab (Van de Geijn *et al.*, 1994). This facility enables a detailed study of the root dynamics of mature plants under field conditions, regardless of the size of the plants and the duration of the experiment (Smit *et al.*, 1994). The ability to repeatedly record root growth on prefixed conditions along glass minirhizotron tubes makes it possible to get detailed information of root production and turnover during the experiment.

In the experiment, *Holcus lanatus* L. a species characteristic of nutrient-rich habitats and *Nardus stricta*, a species characteristic of nutrient-poor habitats are compared. The two species differ in foraging ability and are assumed to differ in root turnover rate. In contrast to *Nardus stricta*, *Holcus lanatus* is able to generate significantly higher root length density in response to nutrient enrichment (Fransen *et al.*, 1998). The assumption that the root turnover rates of the species differ as well is based on the observations that species from nutrient-rich habitats generally display lower tissue lifespans than species from nutrient-poor habitat (Chabot and Hicks, 1982; Reich *et al.*, 1992; Berendse *et al.*, 1999).

Due to the above mentioned differences in root foraging ability and root turnover rates, the species are assumed to depend on different root foraging characteristics for the acquisition of nutrients in heterogeneous environments. *Holcus lanatus*, the species of nutrient-rich habitats, is assumed to rely on a high degree of root morphological plasticity. The high degree of morphological plasticity will enable the acquisition of sufficient amounts of nutrients to offset the nutrient losses due to its high turnover rates. *Nardus stricta*, the species of nutrient-poor habitats, is less morphologically plastic, but is assumed to depend on a large, long-lived root system that remains viable under prolonged periods of stress. This viable root system will enable the species to instantaneously increase its nutrient uptake kinetics in response to nutrient enrichment (Grime *et al.*, 1986; Crick and Grime 1987; Grime *et al.*, 1991).

The long-term merits of both root foraging strategies were determined by comparing shoot biomass production in heterogeneous treatments with shoot biomass production in homogeneous treatments (both nutrient-rich and nutrient–poor) in overall nutrient-poor and overall nutrient-rich environments after two growing seasons. The relative difference, or contrast (Kotliar and Wiens 1990), between the nutrient-rich and nutrient-poor side in the heterogeneous environments was the same under both overall levels of nutrient availability. Under the overall high level of nutrient availability, *Holcus lanatus* may acquire sufficient nutrients, due to its high degree of root proliferation, to offset its high nutrient losses. However, under the overall low level of nutrient availability, the gains in terms of nutrient uptake of selective root placement may be smaller, and hence, *Holcus lanatus* may not be able to acquire sufficient nutrients to offset its nutrient losses. In these habitats a more rigid root development pattern and a low root turnover rate, as displayed by *Nardus stricta*, may be more beneficial. The following hypotheses were tested:

1) *Holcus lanatus* from nutrient-rich habitats displays a higher selective root placement in heterogeneous environments than *Nardus stricta* from nutrient-poor habitats, irrespective of the overall nutrient availability of the environment.
2) *Nardus stricta* from nutrient-poor habitats displays a longer root life span than *Holcus lanatus* from nutrient-rich habitats, irrespective of the overall nutrient availability of the environment.
3) *Holcus lanatus* from nutrient-rich habitats produces relatively more biomass in heterogeneous environments under overall high nutrient availability than *Nardus stricta* from nutrient-poor habitats, because the increased nutrient acquisition due to its higher degree of root morphological plasticity exceeds its nutrient losses due its higher root turnover.
4) *Nardus stricta* from nutrient-poor habitats produces relatively more biomass in heterogeneous environments under overall low nutrient availability than *Holcus lanatus* from nutrient-rich habitats, because its lower nutrient losses due to its lower root turnover rates offsets its reduced nutrient gain by its lower level of morphological plasticity.

Material and methods

Species

In the experiment *Holcus lanatus* L. and *Nardus stricta* L. were used to assess the long-term growth effects of different root foraging strategies. *Holcus lanatus* L. is a fast-growing species (potential RGR = 1.56 week $^{-1}$ (Grime and Hunt, 1975) that is characteristic of nutrient-rich habitats. *Nardus stricta* L. is a slow-growing species (potential RGR = 0.71 week $^{-1}$ (Grime and Hunt, 1975)), characteristic of nutrient-poor habitats. The species are hereafter referred to by their generic names.

The original plants (i.e. group of tillers) of both species were collected in autumn 1994 at different sites in a former agricultural grassland area along the Anlooër Diepje, a brook in the 'Drentse Aa' Nature Reserve (53°N, 6°40'E) (see Bakker 1989). Plants taken from different fields are genotypically different. The nutrient availability of the selected sites differed (Olff et al., 1994), because the application of fertiliser to the sites was stopped in different years (Olff et al., 1990). The individuals of each species used in this study were isolated from plants species that were propagated from the original field material in a heated greenhouse with supplemental lighting from high-pressure sodium lamps (Philips SON-T 400W) giving a photoperiod of 12h. Two genotypes of each species were used.

The Wageningen Rhizolab facility and the experimental design

The Wageningen Rhizolab (Smit *et al.*, 1994; Van de Geijn et al., 1994) enables the detailed study of the root development pattern of mature plants during several growing seasons. Four watertight compartments (1.25m×1.25m×2.00m; width×length×depth) were divided with use of PVC-partitioning into 13 square-shaped sub-compartments (0.28m×0.28m×1.00m, width×length×depth). The joints between the different PVC-parts were sealed with plasticine (Rhiwa-Hartomex B.V., The Netherlands).

The soil profile in each of the compartments consisted of a top layer of 1m that was filled with nutrient-rich and nutrient-poor substrate, and a 1-m subsoil layer of coarse sand

(Fig 1). Each substrate consisted of a mixture of humus-rich black soil (4.1% organic matter, pH-KCl 4.5) and coarse sand. To avoid root penetration into the subsoil layer, this layer was covered with a black PVC sheet (3mm thickness) in which a central hole was created for drainage.

In the top layer of each compartment two substrates were applied in three bands of 40cm width (Fig 1). A compartment consisted either of 2 nutrient-rich bands and 1 nutrient-poor band, or of 1 nutrient-rich band and 2 nutrient-poor bands. In this array, each compartment contained sub-compartments that were either homogeneously nutrient-rich or poor, or heterogeneous. In the heterogeneous sub-compartments, plant roots could freely penetrate into both substrates.

In two of the four compartments nutrient-rich soil consisted of 80% humus-rich black soil (N-mineral 16.3 mg kg^{-1}, 1M KCl-extraction) and nutrient-poor soil consisted of 20% humus-rich black soil (N-mineral 8.8 mg kg^{-1}, 1M KCl-extraction). This combination is hereafter referred to as the overall high level of nutrient availability. In the other two compartments nutrient-rich soil consisted of 20% humus-rich black soil (N-mineral 8.8 mg kg^{-1}, 1M KCl-extraction) and nutrient-poor soil consisted of 5% humus-rich black soil (N-mineral 4.6 mg kg^{-1}, 1M KCl-extraction). This combination is hereafter referred to as the overall low level of nutrient availability. Hence, the overall level of nutrient availability differs between the compartments, but the patch contrast (Kotliar and Wiens, 1990) or relative heterogeneity (Li & Reynolds 1995) is the same (80:20 and 20:5) in each compartment.

The compartments were filled manually (bulk soil density 1.3 kg dm^{-3}) and capacitance moisture sensors, ceramic suction cups (∅ 2.2cm, length 5.5cm) and glass minirhizotron tubes (∅ 6cm, length 1.30m) were installed at fixed places during the filling of the compartments. The compartments were covered with gravel and an automatic drip installation was installed. The automatic drip installation that regulated water gifts based on measured soil water content, insured constant soil water content.

Four minirhizotron tubes were installed horizontally at a depth of 25cm (Fig 1). The minirhizotron tubes penetrated the PVC-partitioning, and the joints between the PVC-parts and the tubes were sealed with plasticine. The open ends of the tubes protruded via plastic cuffs through the wooden panel into the corridor. The tubes were filled with isolation foam and the open ends of the tubes were covered with a metal can to prevent possible effects of temperature differences between the glass tubes and the bulk soil or of light.

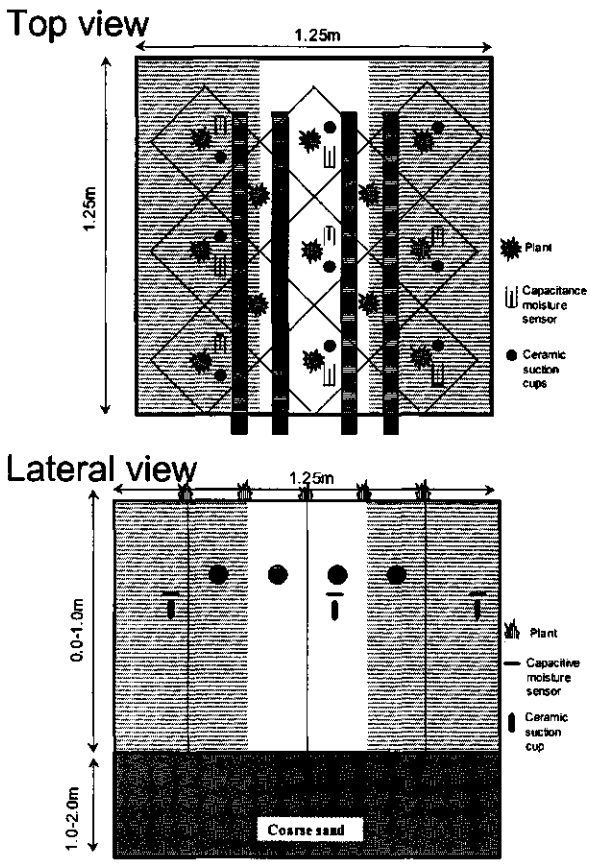

Figure 1. Top and lateral view of the experimental set-up of two of the four compartments used in the Wageningen Rhizolab. The compartments were divided by PVC-partitioning into 13 square-shaped sub-compartments and the top layer (0-1m) was filled with two soil substrates, such that each compartment consisted of homogeneous nutrient-poor, heterogeneous and homogeneous nutrient-rich sub-compartments. Minirhizotron tubes were installed at 25cm depth. Capacitance moisture sensors and ceramic suction cups, installed at a depth of 30cm, were used to measure volumetric soil moisture content and temperature, and to extract soil moisture for nutrient analyses weekly.

Individual plants of each species were divided over the different compartments and sub-compartments, such that each treatment was replicated at least four times. Plants were grown for two growing seasons. In March of the second year, slow-release fertiliser (N:P:K = 13:13:13; release time = 3-4 Months; Osmocote Plus, Scotss Europe BV) was added to the plants to re-establish the initial patch contrast between the nutrient-rich and nutrient-poor soil in the heterogeneous treatment. The amount of fertiliser added to the homogeneous and heterogeneous treatments corresponded with the initial nitrogen content (1M KCl-extraction) of the different soil substrates to a depth of 1m.

Measurements

Biweekly root observations were made in each minirhizotron tube with a video camera at fixed positions. In winter root observations were made once a month. For specific details about the video observation system in the Wageningen Rhizolab, see Smit et al. (1994). Of each individual plant, six root observation positions, located in the centre of the sub-compartment, were monitored during the experiment. Of plants growing in the heterogeneous sub-compartments, six root observations were monitored in both the nutrient-rich and the nutrient-poor side. Each root observation position consists of an area of 14×18mm along the upper side of the tubes.

Volumetric soil moisture content and temperature of 12 sub-compartments in each compartment were measured weekly with the installed capacitance moisture sensors. In the heterogeneous sub-compartments, the volumetric soil moisture content and temperature of only one of the two substrates (i.e. nutrient-rich or nutrient-poor) was measured. At the same time soil solution was extracted from each sub-compartment with ceramic suction cups. The NO_3^- concentration in the extracted soil water was measured using a continuous flow analyser (Skalar, The Netherlands). In the heterogeneous sub-compartments, soil moisture was again extracted from only one of the two substrates.

The plants were harvested above ground at the end of each growing season. In addition, *Holcus* L. plants had to be clipped twice at a height of 10cm during the growing seasons to prevent over-shading of leaves of neighbouring plants. The clipped plant material was collected and used in the calculation of the shoot biomass production of *Holcus* . Shoot biomass was determined after drying at 70°C for at least 48h.

At the end of the experiment, two soil-cores in each of the sub-compartments were taken at a depth of 0-20cm and at a depth of 30-50cm with an auger (∅ 5cm). In the heterogeneous sub-compartments one soil-core in each of the two substrates (i.e. nutrient-rich or –poor) was taken. Roots in these soil-cores were washed clean of soil particles and root length and root biomass was determined. Root biomass was determined after drying at 70°C for at least 48h.

Image analysis

Minirhizotron video-images (14×18mm) were digitised, and contrast and brightness were optimised automatically for each image with image analysis software. Because video images displayed a small overlap 2mm of the lower part of the image was removed. The root-images were stored as frames of 600×772 pixels (256 grey levels). For the analysis of root length, three adjacent images were combined, resulting in an image of 36×18mm surface area at the minirhizotron tube. Roots had to be traced manually due to the large overlap in grey levels between roots and soil particles. The length of trace-lines was automatically converted to actual root length. The sequential root observations enabled the determination of root development and root lifespan during the experiment. However, a major problem in assessing root lifespan is the definition of root death. Roots may exhibit signs of necrosis in portions of its length while other portions remain healthy (Eissenstat and Yanai, 1997). Also during this experiment roots became gradually darker and narrower. However, roots were only classified as dead if they became invisible since roots may continue to absorb water and nutrients after death of the epidermical and cortex cells (Eissenstat and Yanai, 1997).

Statistical analysis

Analyses of soil data, visible root length, root turnover and shoot biomass

Soil temperature, soil moisture content and soil NO_3^- availability were analysed with a three way ANOVA using GLM (SPSS 1995) with species, overall nutrient level and nutrient distribution nested within overall nutrient level as main factors, and date as a co-variable.

Minirhizotron visible root length data were analysed with a three way ANOVA using GLM (SPSS 1995) with species, overall nutrient level and nutrient distribution nested within overall nutrient level as main factors, and date as a co-variable. At the end of each growing season (i.e. October 1996 and September 1997) analyses of variance of visible root length were carried out separately for each species×nutrient level combination.

Root turnover is determined by following the presence of roots from the initial root cohort produced that appeared in the minirhizotron video images between the start of the

experiment (29 May 1996) and 11 July 1996. The percentage survival was used as an indication of root turnover rate. Cohort analysis (Pyke and Thompson, 1986; Pregitzer *et al.*, 1995) was used to quantify treatment effects on root turnover. Effects of nutrient distribution and overall nutrient availability were determined using a Gehan-Wilcoxon nonparametrics test (SPSS 1995) appropriate for survivorship data in which not all individuals disappear during the experiment (Pyke and Thompson, 1986).

Shoot biomass produced in the second growing season is used to assess the long-term effects of root foraging. Shoot biomass was analysed with a three way ANOVA using GLM (SPSS, 1995). A posteriori tests within each combination of species and nutrient level were carried out using Tukey's HSD-test.

Analysis of root foraging responses

Like all measurements of phenotypic plasticity, selective root placement can be analysed at a common point in time or at a common plant size (Coleman *et al.*, 1994). Analysis of selective root placement at a common point in time are important in relation to 'real time' processes, such as plant-plant interactions, and for the analysis of seasonal patterns (Coleman *et al.*, 1994). Analysis of a selective root placement at a common plant size is important to assess the functional adjustment of the foraging response (Fransen *et al.*, 1999). Plants display ontogenetic drift in many phenotypic traits, especially in traits related to any aspect of plant size, and as a result proportional biomass distribution over the different plant parts is rarely constant for extended periods (Evans, 1972). To distinguish between the functional adjustment of a phenotypic response and ontogenetic drift, differences in biomass partitioning in response to nutrient heterogeneity need to be evaluated at a common plant size (Coleman and McConnaughay, 1995; Coleman et al., 1994; Evans, 1972). Therefore, in the experiment selective root placement is not only given as root biomass production in response to nutrient heterogeneity, but is also expressed per unit shoot biomass to account for differences in plant size between the treatments.

Root biomass production within the soil-cores is analysed with a three-way ANOVA, using GLM (SPSS 1995) with species, overall nutrient level and nutrient distribution nested within overall nutrient level as main factors. To account for plant size dependent variation in root biomass, ln-transformed shoot biomass was used as a co-variable (cf. Klinkhamer *et al.*,

1990). Root biomass data from both soil-cores taken within a homogeneous sub-compartment were nested to avoid pseudo-replication. To determine the root foraging ability of the species both at a common point in time and at a common size, three a priori comparisons of root biomass production were carried out within each combination of species and nutrient level: (1) nutrient-rich to nutrient-poor within the heterogeneous treatment, testing whether species are able to produce more roots in the nutrient-rich side than in the nutrient-poor side of the heterogeneous treatment, (2) heterogeneous nutrient-rich to homogeneous nutrient-rich, and (3) heterogeneous nutrient-poor to homogeneous nutrient-poor. The latter two comparisons (i.e. 2 and 3) test whether the root placement pattern resulted from localised growth responses and to what extent integration within the root system had significant effects. In general, root growth is closely co-ordinated among different parts of the root systems, and increased root growth in nutrient-rich patches may occur at the expense of root growth elsewhere in the root system (Gersani and Sachs, 1992; Robinson, 1994; Robinson, 1996; Robinson and Van Vuuren, 1998). Hence, if integration within the root system of the species occurs, root biomass in the nutrient-rich side in the heterogeneous treatment will be higher than root biomass in the same volume of the homogeneous nutrient-rich treatment, and simultaneously, root biomass in the nutrient-poor side of the hetogeneous treatment will be lower than the root biomass in the same volume of the homogeneous nutrient-poor treatment. However, plants in the different treatments will be of different size when compared, due to the differences in nutrient availability between the treatments. Therefore, as explained above, it is necessary to correct for plant size in the analyses. Each pair of comparison matches the assumption of orthogonality and normal F-tests without corrections were carried out.

Analysis of root foraging merits against a 'null-model'

In the experiment, plants were grown in homogeneous nutrient-rich and –poor environments and in heterogeneous environments under two overall levels of nutrient availability during two growing seasons. To determine the merits of root foraging responses in terms of biomass, we developed a 'null-model' to predict the amount of shoot biomass produced by a species in the heterogeneous treatment based on its biomass production in the homogeneous nutrient-rich and nutrient–poor treatment. Note: The below-defined 'null-model' is only true if the sizes of the nutrient-rich and –poor patches comprise 50% of the available area.

Our 'null-model' of biomass production states that biomass production in the heterogeneous treatment will be exactly intermediate between biomass production in the homogeneous nutrient-rich and homogeneous nutrient-poor treatment. This hypothesis holds if a species acquires equal amounts of nutrients per unit plant size from each side in the heterogeneous treatment as from the same soil volume in the coinciding homogeneous treatments, and thus if root density (length or biomass) per unit plant size and uptake rate per unit root (length or biomass) in each side are equal to those in the coinciding homogeneous treatments. Hence, a species must be able to forage, i.e. selectively increase root density and nutrient uptake rate per unit plant size (cf. Fransen *et al.*, 1999), in the nutrient-rich side as compared to the nutrient-poor side in the heterogeneous treatment to produce the amount of biomass according to the 'null-model'.

If a species is not able to forage selectively for nutrients in the nutrient-rich side of the heterogeneous treatment, but divides root biomass more equally over both sides in the heterogeneous treatment, the species will acquire less nutrients per unit plant size from the nutrient-rich side than from the same volume in the homogeneous nutrient-rich treatment. Consequently, the biomass production in the heterogeneous treatment will be lower than expected based on the 'null-model'.

A species will only be able to produce more biomass than expected based on the 'null-model' if it is able to generate a higher nutrient acquisition per unit plant size from the heterogeneous nutrient-rich side than from the same soil volume in the homogeneous nutrient-rich treatment. One way to achieve this is by a coordinated foraging response that results in the production of relatively more roots in the nutrient-rich side and less in the nutrient-poor side of the heterogeneous treatment.

The relative shoot biomass production of the species in the heterogeneous treatments is determined by testing the actual shoot biomass in the heterogeneous treatment against the 'null-model' of biomass production. Shoot biomass in the heterogeneous treatment under both levels of overall nutrient availability is a priori tested against the shoot biomass production in the coinciding homogeneous (nutrient-rich and –poor) treatments (SPSS, 1995). The a priori test matches the assumption of orthogonality and normal F-tests without corrections were carried out. All data were checked for deviations from normality and for homogeneity of variance prior to analysis and transformed where necessary.

Results

Soil conditions

Soil moisture content (Fig. 2A,B) did not significantly differ between the treatments during the experiment (Table 1). The soil moisture content of the nutrient-rich soil was generally higher than in the nutrient-poor soil under both overall levels of nutrient availability ($P<0.001$, Fig. 2A,B), probably due to a higher organic matter content in the nutrient-rich soil than in the nutrient-poor soil.

Soil temperature varied significantly during the experiment, but the changes in temperature were similar for the two species, the two overall levels of nutrient availability and the nutrient distribution within the overall nutrient levels (Table 1).

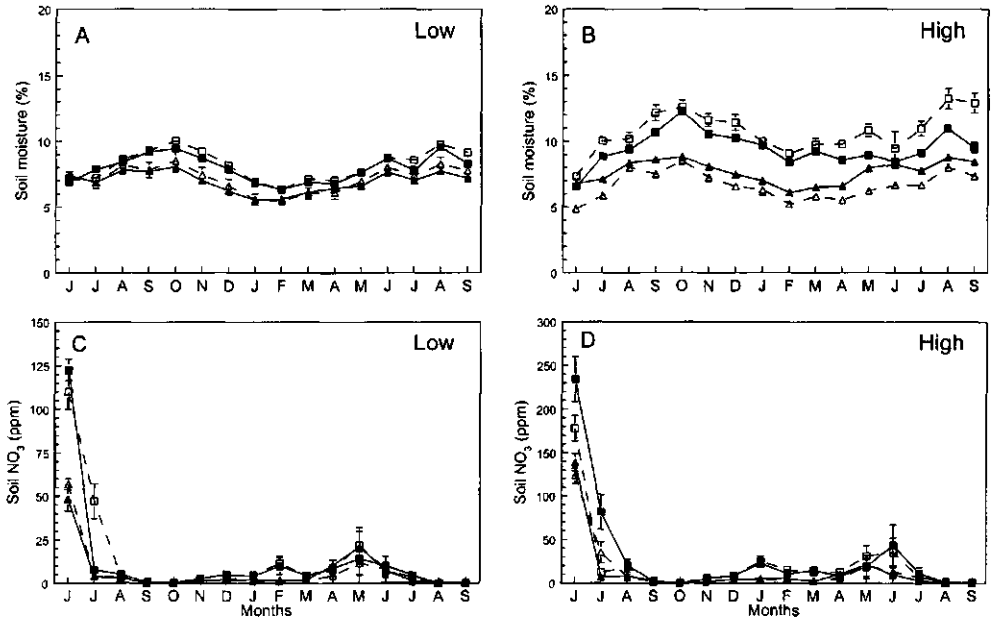

Figure 2. Monthly averages of soil moisture percentage (v/v; A, B) and nitrate content of extracted soil water (ppm; C, D) for the homogeneous nutrient-poor (closed triangles), the heterogeneous nutrient-poor (open triangles), the heterogeneous nutrient-rich (open squares) and the homogeneous nutrient-rich (closed squares) treatments at 30-cm depth under an overall low level of nutrient availability (Low) and an overall high level of nutrient availability (High). Vertical bars indicate 1 SE.

Table 1. Analysis of variance using a general linear model for soil moisture, soil temperature and NO_3-content of the extracted soil water with species, the overall nutrient level of the treatments and the nutrient distribution (i.e. homogeneous nutrient-rich and –poor and heterogeneous nutrient-rich and –poor) nested within the overall nutrient level as main factors and date as a co-variable. Soil moisture data were ln-transformed, temperature and NO_3 data were not transformed prior to analysis. F-values are given. (x=700, 646 and 761 for moisture, temperature and NO_3, respectively)

Effect	df	Moisture	Temperature	NO_3
Date	1, x	1.94 ^{NS}	9.69**	112.12***
Species	1, x	4.86*	0.38 ^{NS}	0.41 ^{NS}
Nutrient Level	1, x	3.59 ^{NS}	0.02 ^{NS}	25.87***
Nutrient distribution within Level	6, x	8.21***	0.12 ^{NS}	6.29***
Spec×Date	1, x	1.44 ^{NS}	0.20 ^{NS}	1.51 ^{NS}
Level×Date	1, x	1.35 ^{NS}	0.01 ^{NS}	13.80***
Nutr. distr. within Level×Date	6, x	0.28 ^{NS}	0.07 ^{NS}	3.31**
Spec×Level	1, x	0.04 ^{NS}	0.01 ^{NS}	0.17 ^{NS}
Spec×Nutr. distr. within Level	6, x	3.21**	0.10 ^{NS}	0.51 ^{NS}
Spec×Level×Date	1, x	0.15 ^{NS}	0.01 ^{NS}	0.04 ^{NS}
Spec×Nutr. distr. within Level×Date	6, x	1.31 ^{NS}	0.02 ^{NS}	0.19 ^{NS}

NS = not significant, *P<0.05, **P<0.01, ***P<0.001

The initial NO_3^--content of the extracted soil water differed significantly (P<0.001) between the overall high level of nutrient availability and the overall low level, but did not differ significantly between the homogeneous treatments and their coinciding sides in the heterogeneous treatments in none of the two overall levels of nutrient heterogeneity. The NO_3^--content of the extracted soil water (Fig. 2C,D) declined significantly over the course of the experiment (Table 1). The decrease in NO_3^--content differed significantly between the two overall levels of nutrient availability, as indicated by the significant level×date interaction (P<0.001; Table 1). The decrease in NO_3^--content was higher in the overall high nutrient level than in the overall low nutrient level. Also within each of the overall nutrient levels, the decrease in NO_3^--content differed significantly between the different treatments, as indicated by the significant nutrient distribution within nutrient level×date interaction (P<0.01; Table 1). The decrease in NO_3 content was more pronounced in the nutrient-rich soil in both the heterogeneous and homogeneous treatments than in the nutrient-poor soil of both treatments under both levels of overall nutrient availability.

Root development pattern

The dynamics of the root development pattern is assessed by the root length visible along the minirhizotron tubes (Fig. 3; Table 2). In general, root length of both species increased during the first growing season, decreased during winter and increased rapidly during spring of the second year. However, the decrease in root length during winter was much more pronounced for *Holcus* than for *Nardus*, but the latter species had produced far less roots at the end of the first growing season than the former species.

At the end of the first growing season (i.e. October 1996), root length of *Holcus* (Fig. 3A,B) was generally higher under the overall high level of nutrient availability than under the

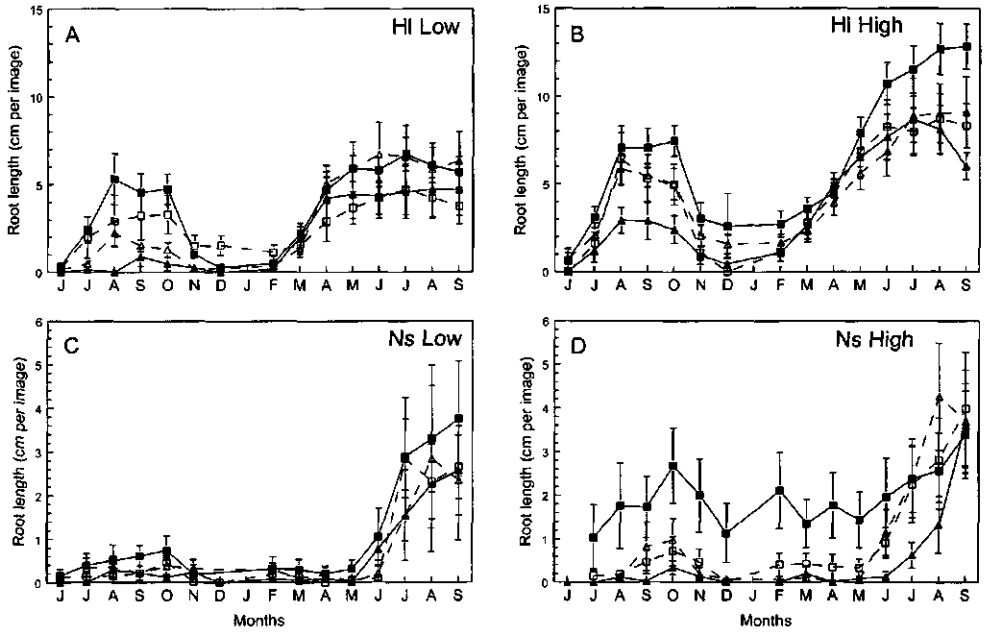

Figure 3. Average root length per image (36×18mm) for *Holcus lanatus* (Hl) and *Nardus stricta* (Ns) in the homogeneous nutrient-poor treatment (closed triangles), the nutrient-poor side of the heterogeneous treatment (open triangles) the nutrient-rich side of the heterogeneous treatment (open squares) and the homogeneous nutrient-rich treatment (closed squares) under an overall low level of nutrient availability (Low) and an overall high level of nutrient availability (High) as determined by minirhizotron observations. Vertical bars indicate 1 SE.

overall low level of nutrient availability. However, in none of the two overall levels of nutrient availability did visible root length differ significantly ($P>0.05$) between the nutrient-rich and nutrient-poor side of the heterogeneous treatment. However, visible root length in the nutrient-poor side of the heterogeneous treatment was significantly higher than in the homogeneous nutrient-poor treatment in the overall high level of nutrient availability. At the end of the second growing season (i.e. September 1997), still no significant differences ($P>0.05$) in visible root length of *Holcus* could be detected between the two sides of the heterogeneous treatments in none of the two overall levels of nutrient availability. However, visible root length in the nutrient-rich side of the heterogeneous treatment was significantly ($P<0.05$) lower than in the homogeneous nutrient-rich treatment under the overall high level of nutrient availability.

For *Nardus* (Fig. 3C,D) no differences in root length could be detected between the sides in the heterogeneous treatment or between the homogeneous and heterogeneous treatments, neither at the end of the first growing season nor at the end of the second.

Table 2. Analysis of variance using a general linear model for root length visible along the minirhizotron tubes with species, the overall nutrient level of the treatments and the nutrient distribution (i.e. homogeneous nutrient-rich and –poor and heterogeneous nutrient-rich and –poor) nested within the overall nutrient level as main factors and date as a co-variable. Data were ln-transformed prior to analysis. F-values are given.

Effect	df	Root length
Date	1	185.74***
Species	1	41.61***
Nutrient Level	1	0.40 ^{NS}
Nutrient distribution within Level	6	4.90 ***
Spec×Date	1	0.52 ^{NS}
Level×Date	1	0.97 ^{NS}
Nutr. distr. within Level×Date	6	2.35*
Spec×Level	1	1.02 ^{NS}
Spec×Nutr. distr. Within Level	6	3.08**
Spec×Level×Date	1	0.28 ^{NS}
Spec×Nutr. distr. Within Level×Date	6	2.98**
Error	1282	

NS = not significant, *P<0.05, **P<0.01, ***P<0.001

Selective root placement

After two years, root length present within the soil-cores differed significantly between species and nutrient distribution within nutrient level (Table 3). The non-significant interaction species×nutrient distribution within nutrient level (Table 3) indicated that overall *Holcus* and *Nardus* responded similarly in terms of root length production in response to nutrient heterogeneity.

Table 3. Analysis of variance using a general linear model for root length and root biomass produced within soil-cores at the end of the second growing season. Species, the overall nutrient level and the nutrient distribution (i.e. homogeneous nutrient-rich and –poor and heterogeneous nutrient-rich and –poor) nested within the overall nutrient level were used as main factors. Shoot biomass is used as a co-variable in the analysis of root biomass production per unit shoot biomass. Length and biomass data were ln-transformed prior to analysis. F values are given.

Effects	df	Root length	Root DW	Size-corrected Root DW
Shoot DW	1	---	---	6.43*
Species	1	343.94***	16.67***	0.04 ^{NS}
Nutrient Level	1	0.74 ^{NS}	14.97***	1.52 ^{NS}
Nutrient distribution within Level	6	3.46**	3.03*	0.22 ^{NS}
Spec×Shoot DW	1	---	---	0.01 ^{NS}
Level×Shoot DW	1	---	---	0.67 ^{NS}
Nutr. distr. Within Level×Shoot DW	6	---	---	0.25 ^{NS}
Spec×Level	1	0.04 ^{NS}	8.44**	0.22 ^{NS}
Spec×Nutr. distr. within Level	6	0.59 ^{NS}	0.97 ^{NS}	0.36 ^{NS}
Spec×Level×Shoot DW	1	---	---	0.23 ^{NS}
Spec×Nutr. distr. Within Level×Shoot DW	6	---	---	0.35 ^{NS}
Error		52	49	36

NS = not significant, *P<0.05, **P<0.01, ***P<0.001

The data on root length differed from the data on root biomass production within the soil-cores with regard to the effect of overall nutrient level. Overall nutrient level had no significant effect on root length within the soil-cores, but significantly affected root biomass production (Table 3). This difference must be caused by a significant alteration of the specific

root length by the species in response to the overall level of nutrient availability. However, it is more important that within each overall level of nutrient availability, root length and root biomass data displayed exactly the same pattern.

Under the overall low level of nutrient availability, root biomass production of *Holcus* in the nutrient-rich side of the heterogeneous treatment did not differ significantly (P=0.435) from the root biomass in the nutrient-poor side of that treatment (Fig. 4A). However, under the overall high level of nutrient availability, *Holcus* produced significantly (P=0.048) more root biomass in the nutrient-rich side of the heterogeneous treatment than in the nutrient-poor side of that treatment (Fig. 4B).

To test whether this selective increase in root biomass in the nutrient-rich side in the heterogeneous treatment under the overall high nutrient level was similar to the root biomass under homogeneous nutrient-rich conditions, it is necessary to correct root biomass production within the soil-cores for differences in plant size. Larger plants may generate relatively larger differences in root biomass between nutrient-rich and nutrient-poor patches. Root biomass production per unit soil volume of the species in the homogeneous treatments was indeed significantly positively correlated with shoot biomass (r^2=0.29, n=39, P=0.001).

Analysis showed that the root biomass production of *Holcus* in the nutrient-rich side of the heterogeneous treatment under the overall high nutrient level was significantly (P=0.017) higher than in the homogeneous nutrient-rich treatment (Fig. 4E). However, root biomass production per unit shoot biomass in the nutrient-poor side in the heterogeneous treatment did not differ significantly (P=0.173) from the homogeneous nutrient-poor treatment (Fig. 4E). Apparently, *Holcus* increased its root density in the nutrient-rich side in the heterogeneous treatment, but not at the expense of the roots placed in the nutrient-poor side.

Nardus was not able to produce significantly more root biomass in the nutrient-rich side of the heterogeneous treatment than in the nutrient-poor side, neither in the overall low level (P=0.906; Fig. 4C), nor in the overall high level of nutrient availability (P=0.399; Fig. 4D). However, when root biomass production was analysed per unit shoot biomass, *Nardus* tended to produce more root biomass in the nutrient-rich side of the heterogeneous treatment than in the homogeneous nutrient-rich treatment, under both the overall low level (P= 0.090); Fig. 4G) and the overall high level of nutrient availability (P= 0.171; Fig. 4H).

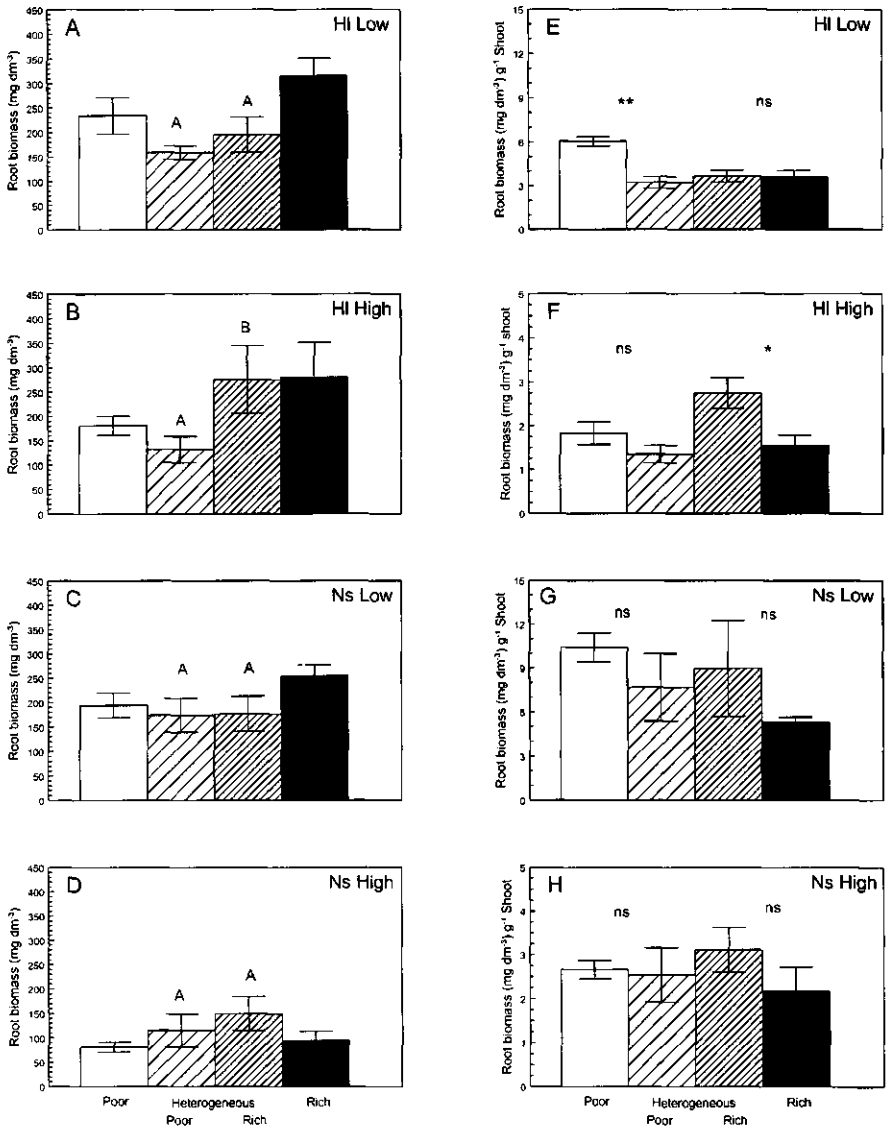

Figure 4. Root dry weight production (A-D) and root dry weight production per gram shoot biomass (E-H) within in the soil cores taken for *Holcus lanatus* (Hl) and *Nardus stricta* (Ns) under an overall low level of nutrient availability (Low) and an overall high level of nutrient availability (High) within the homogeneous nutrient-poor treatment (open bars), the nutrient-poor side of the heterogeneous treatment (wide-hatched bars), the nutrient-rich side of the heterogeneous treatment (narrow-hatched bars) and the homogeneous nutrient-rich treatment (closed bars). Data are means ± SE (n=4 or 5). For root biomass (A-D) bars with the same letter within each species×nutrient level combination are not significantly different (P>0.05). For root biomass per gram shoot biomass (E-F) asterisks within each species×nutrient level combination indicate significant differences between a side of the heterogeneous treatment and the corresponding homogeneous treatment. *P<0.05, **P<0.01, ns not significant.

Root longevity

Root longevity differences between *Holcus* and *Nardus* are determined using only roots present in the homogeneous nutrient-rich treatments, because only this treatment contained roots of *Nardus* that were produced in the initial root cohort. Cohort survivorship analysis showed that root longevity was only marginally different between *Holcus* and *Nardus* (Fig. 5; P=0.072), probably due to the limited number of roots of *Nardus* (n=7) present in the initial cohort.

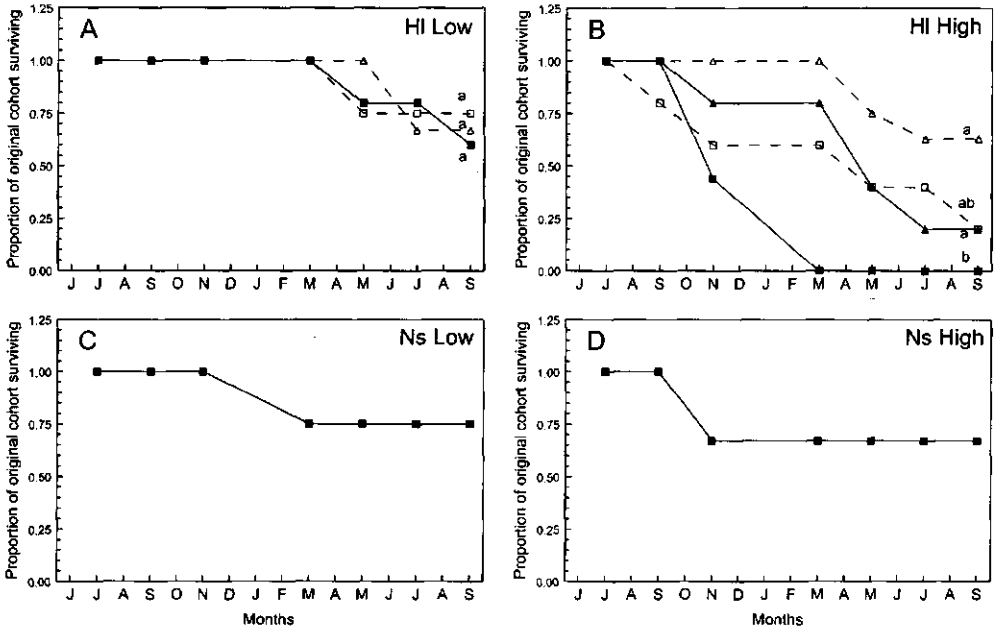

Figure 5. Root survival curves for the root cohort produced before 11 July 1996 for *Holcus lanatus* (Hl) and *Nardus stricta* (Ns) in the homogeneous nutrient-poor treatment (closed triangles), the nutrient-poor side of the heterogeneous treatment (open triangles) the nutrient-rich side of the heterogeneous treatment (open squares) and the homogeneous nutrient-rich treatment (closed squares) under an overall low level of nutrient availability (Low) and an overall high level of nutrient availability (High). Data are means ± SE (n=3-9). Lines with the same letters within each species×nutrient level combination are not significantly different (Gehan-Wilcoxon test, P>0.05).

Roots of *Holcus* lived significantly (P=0.013) longer under the overall low level of nutrient availability (Fig. 5A) than under the overall high level of nutrient availability (Fig. 5B). Strikingly, under the overall high level of nutrient availability (Fig. 5B), root longevity of *Holcus* was significantly higher in the nutrient-poor soil in the homogeneous nutrient-poor and heterogeneous treatment than in the nutrient-rich soil in the homogeneous nutrient-rich treatment (P=0.024 and P=0.0003, respectively).

Root longevity of *Nardus* did not differ between the two overall levels of nutrient availability (Fig. 5C,D; P=0.244), but again only a very limited number (n=7)) of roots were present in the initial cohort, reducing the power of the statistical test.

Shoot biomass production

Holcus produced significantly (P<0.05) more shoot biomass in the homogeneous nutrient-rich treatment than in the homogeneous nutrient-poor treatment under both overall levels of nutrient availability (Fig. 6A,B).

The long-term merits of root foraging in this experiment were determined by comparing the shoot biomass production of the species in the heterogeneous treatment relative to the shoot biomass in the homogeneous nutrient-rich and -poor treatments. Our defined 'null-model' predicts that biomass production in the heterogeneous treatment is exactly intermediate between the biomass production in the homogeneous treatments. Results according to the 'null-model' are expected if plants are able to selectively increase root density in the nutrient-rich side in the heterogeneous treatment. The total root biomass production of the species after two years was estimated, but since the results of total biomass production remained qualitatively the same as the results based on total shoot biomass, we only display the results of total shoot biomass.

After two growing seasons, the shoot biomass production of *Holcus* in the heterogeneous treatment under the overall low level of nutrient availability was lower on average, though not significant (P=0.432), than the expected shoot biomass based on the 'null-model' (Fig. 6A). However, under the overall high level of nutrient availability shoot biomass production of *Holcus* in heterogeneous treatment was a lot lower than the expected shoot biomass based on the 'null-model', but, due to the low number of replicates, was only

marginally (P=0.072) significant (Fig. 6B). Noteworthy is that the total shoot biomass of *Holcus* in the heterogeneous treatments did not differ significantly from the shoot biomass production in the homogeneous nutrient-poor treatment, in none of the two overall levels of nutrient availability (Fig. 6A,B)

In the homogeneous treatments, *Nardus* produced more shoot biomass in the nutrient-rich treatment than in the nutrient-poor treatment under both overall levels of nutrient availability. However, the increase in shoot biomass production was only significant (P<0.05) under an overall low level of nutrient availability (Fig. 6C,D).

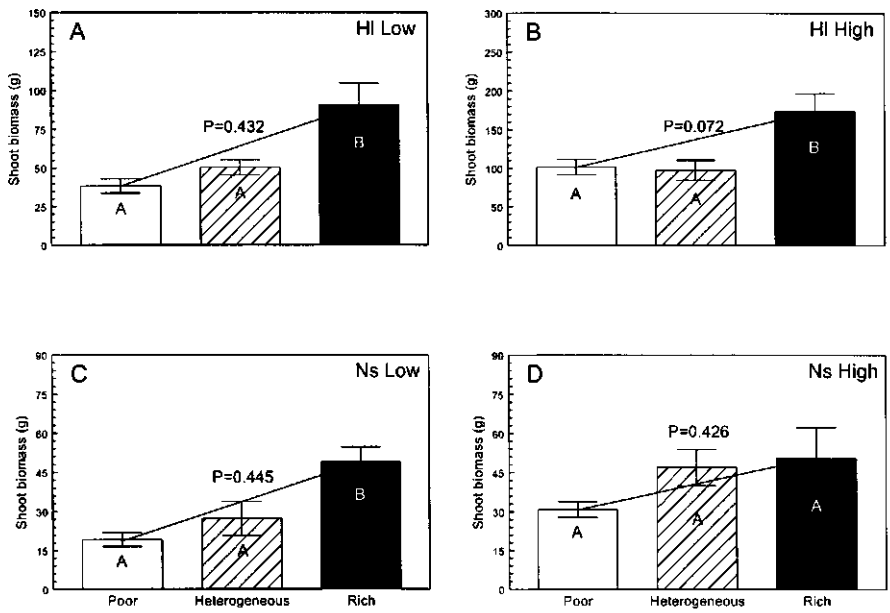

Figure 6. Shoot biomass production in 1997 of *Holcus lanatus* (Hl) and *Nardus stricta* (Ns) in the homogeneous nutrient-poor treatment (open bars), the heterogeneous treatment (hatched bars) and the homogeneous nutrient-rich treatment (closed bars) under an overall low level of nutrient availability (Low) and an overall high level of nutrient availability (High). Data are means ± SE (n=4 or 5). Within each species×nutrient level combination, bars with the same letter are not significantly different (P>0.05). The difference between the actual shoot biomass production in the heterogeneous treatment and the 'null-model' of biomass production (solid line) is indicated by the P-values. The 'null-model' of biomass production in the heterogeneous treatment is based on the shoot biomass production of the species in the homogeneous nutrient-rich and nutrient-poor treatments.

After two growing seasons, the shoot biomass production of *Nardus* in the heterogeneous treatment under the overall low level of nutrient availability did not significantly (P=0.445) differ from the expected shoot biomass based on the 'null-model' (Fig. 6C). However, under the overall high level of nutrient availability shoot biomass production of *Nardus* in heterogeneous treatment tended to be higher than the expected shoot biomass based on the 'null-model', but this difference was not significant (P=0.426; Fig. 6D). Note, the total shoot biomass of *Nardus* in the heterogeneous treatment did also not differ significantly from the shoot biomass production in the homogeneous nutrient-poor treatment, in none of the two overall levels of nutrient availability.

Table 4. Analysis of variance using a general linear model for shoot biomass with species, the overall nutrient level of the treatments and the nutrient distribution (i.e. homogeneous nutrient-rich and –poor and heterogeneous nutrient-rich and –poor) nested within the overall nutrient level as main factors. Data were ln-transformed prior to analysis. F values are given.

Effects	df	Shoot biomass
Species	1	92.49***
Nutrient Level	1	37.57***
Nutrient distribution within Level	4	10.96***
Species×Nutrient Level	1	5.27*
Species×Nutrient distr. within Level	4	0.88 ^{NS}
Error	40	

NS = not significant, *P<0.05, **P<0.01, ***P<0.001

Discussion

In this experiment, the long-term benefits of root foraging responses of *Holcus lanatus* and *Nardus stricta*, two perennial grass species characteristic of habitats that differ in nutrient availability, were examined. The experiment lasted for two growing seasons and is, to our knowledge, the first foraging experiment that lasted long enough to include effects of differences in root turnover. The root foraging ability of the species in nutrient-rich and nutrient–poor habitats was examined by creating heterogeneous environments under two overall levels of nutrient availability. The long-term benefits of root foraging for the two

species were assessed by comparing the shoot biomass production of the species in the heterogeneous treatment against a 'null-model' of biomass production. The 'null-model' predicts that biomass production in the heterogeneous treatment is exactly intermediate between the biomass production in the homogeneous treatments. Results according to the 'null-model' are to be expected if plants are able to selectively increase nutrient acquisition from the nutrient-rich side in the heterogeneous treatment to the extent that the roots acquire nutrients from the same volume in the homogeneous nutrient-rich treatment.

We hypothesised that due to their differential root foraging strategies *Holcus* and *Nardus* would differ in their ability to benefit from nutrient heterogeneity under an overall high and an overall low level of nutrient availability. *Holcus* was expected to produce relatively more biomass in heterogeneous environments under overall high nutrient availability than *Nardus*, because the increased nutrient acquisition due to its higher degree of root morphological plasticity was expected to exceed its nutrient losses due its higher root turnover. *Nardus* was expected to produce relatively more biomass in heterogeneous environments under overall low nutrient availability than *Holcus*, because its lower nutrient losses due to its lower root turnover rates were expected to offset its reduced nutrient gain by its lower level of morphological plasticity.

The results show that, in contrast to our hypotheses, *Holcus* tended to produce less shoot biomass than expected in the heterogeneous treatments under both overall levels of nutrient availability after two years. Shoot biomass of *Holcus* in the heterogeneous treatment was similar to the shoot biomass in the homogeneous nutrient-poor treatment in both of the two overall levels of nutrient availability. In contrast, shoot biomass production of *Nardus* in the heterogeneous treatment was more similar to the expected shoot biomass in both of the two overall levels of nutrient availability.

According to the defined 'null-model', a species would produce less shoot biomass in the heterogeneous treatment than expected based on the 'null-model' if it is was not able to selectively forage for nutrients within the heterogeneous treatment. But, *Holcus* was able to significantly increase root biomass in the nutrient-rich side compared to the nutrient-poor side under the overall high level of nutrient availability and still it tended to produce less biomass in the heterogeneous treatment than expected.

This surprising result can only be explained if the long-term rewards of root proliferation are lower than expected based on the short-term foraging experiments carried out

so far. There are two reasons that may have reduced the potential benefits of root proliferation in this long-term experiment. First, high nutrient losses due to root turnover reduce the net benefits of root proliferation. Second, patch depletion limits the potential benefits of root proliferation in heterogeneous environments (Van Vuuren *et al.*, 1996; Fransen *et al.*, 1998) and the negative effect may be more pronounced in long-term experiments. Below the effects of root turnover and patch depletion on the potential benefits of root proliferation are addressed successively.

Roots of *Holcus* tended to live shorter than roots of *Nardus*. In general, species from inherently nutrient-rich habitats have shorter root life spans (Ryser, 1996; Schläpfer and Ryser, 1996), resulting in higher nutrient losses than species from inherently nutrient-poor habitats (Vázquez de Aldana *et al.*, 1996). However, the root longevity of *Holcus* appeared to be plastic: root longevity under nutrient-poor conditions was significantly higher than under nutrient-rich conditions. This plasticity in root longevity of *Holcus* confirms other studies that show that root longevity decreases with increasing nutrient availability (Gross *et al.*, 1993; Pregitzer *et al.*, 1995). The root longevity results show that the lower biomass production of *Holcus* in the heterogeneous treatment compared to the homogeneous treatments can not be attributed to higher nutrient losses due to higher root turnover rates in the heterogeneous treatment. Root longevity in the nutrient-rich and nutrient-poor side in the heterogeneous treatment tended to be even higher than the root longevity in the coinciding homogeneous treatments.

The only remaining reason for the poor performance of *Holcus* in the heterogeneous treatments is that patch depletion limits the potential benefits of selective root placement. Under the overall high level of nutrient availability, root biomass per unit shoot biomass of *Holcus* in the nutrient-rich side in the heterogeneous treatment was significantly higher than in the homogeneous nutrient-rich treatment. Possibly, *Holcus* allocated more root biomass to the nutrient-rich side in the heterogeneous treatment under the overall high level of nutrient availability than necessary to acquire the available nutrients (cf. Robinson, 1996; Van Vuuren, 1996).

The production of a larger root system in the heterogeneous treatment under the overall high level of nutrient availability enabled *Holcus* to selectively place more roots per unit shoot biomass in the nutrient-rich side than in the nutrient-poor side. The significant increase in root biomass production per unit shoot biomass in the nutrient-rich side in the

heterogeneous treatment under the overall high level of nutrient availability did not significantly reduce the root biomass per unit shoot biomass in the nutrient-poor side. Hence, the increased root biomass must be due to a higher allocation of biomass towards the root system. Inevitably, a higher allocation of available biomass to roots will result in a lower shoot biomass production.

The most surprising result is that *Holcus* tended to produce less biomass than expected based on the 'null-model', even though the species was able to selectively place more roots in the nutrient-rich side in the heterogeneous treatment. This result suggests that a high degree of root morphological plasticity not only has limited benefits for nutrient acquisition in heterogeneous environments (Fransen *et al.,* 1998; Ryel and Caldwell, 1998), but may, in the long-term, even result in a disadvantage compared to species that display a more stable pattern of root development, such as *Nardus*.

Shoot biomass production of *Nardus* in the heterogeneous treatments did not significantly differ from the expected biomass based on the 'null-model, in none of the two overall levels of nutrient availability. Hence, *Nardus* must have acquired equal amounts of nutrients per unit plant size from each side in the heterogeneous treatment as from the same soil volume in the coinciding homogeneous treatments. *Nardus* was able to acquire equal amounts without displaying selective root placement, probably because the root biomass per unit shoot biomass within each side in the heterogeneous did not differ significantly from the root biomass in the coinciding homogeneous treatments.

If selective root placement does not result in the acquisition of more nutrients, and eventually may even result in the production of less biomass in heterogeneous than in homogeneous environments, then why is this response so widespread among plant species? Root physiological plasticity may be even more important for rapid nitrogen uptake prior to patch depletion than root proliferation (Van Vuuren *et al.,* 1996; Ryel and Caldwell, 1998). We have to bear in mind that the ability of plants to respond morphologically to heterogeneity may be critical for its belowground competitive success (Casper and Jackson, 1997). When grown in competition, the costs of over-producing roots in nutrient-rich patches may outweigh the risk of other plants gaining advantage in terms of nutrient acquisition. Recently, it has been shown that, in the short-term, strong root proliferation in nutrient enriched patches indeed confers an ecological advantage when plants are grown in competition (Robinson *et al.,* 1999). In a separate experiment, we determined the long-term consequences of differences

in root foraging ability for the competitive ability of two perennial grass species in heterogeneous environments. The results of this experiment will be described in a future paper (Fransen *et al.*, in prep.).

Acknowledgements

We are very much indebted to F. Berendse and J. Groenwold for their advice with regard to various aspects of the experiment. Special thanks also to J. Blijjenberg and G. Versteeg for their technical assistance during the experiment, and to W. Arp for his help during the image analysis.

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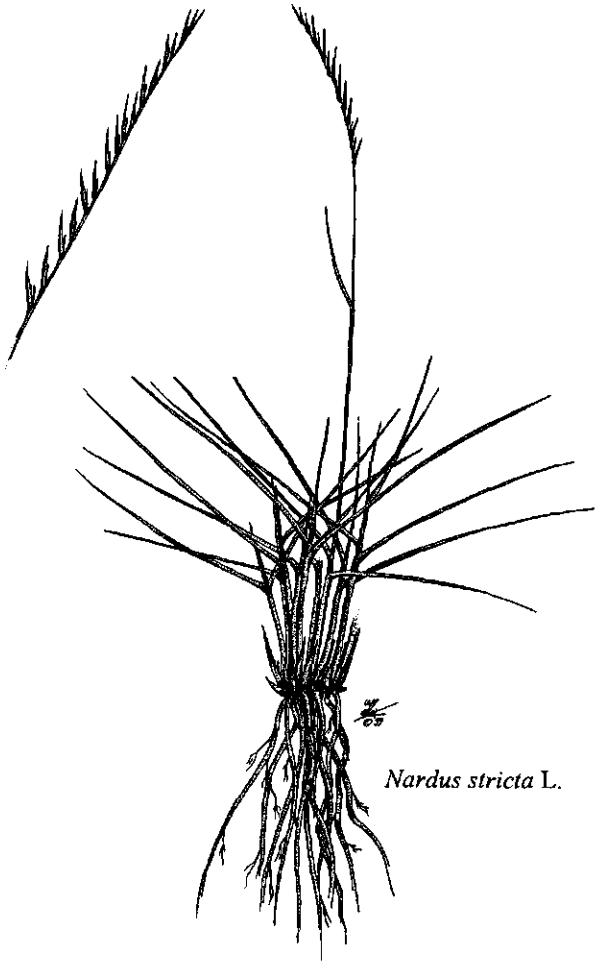
Chapter 6

Nutrient heterogeneity changes competition between populations of *Festuca rubra* L. and *Anthoxanthum odoratum* L.

Bart Fransen, Hans de Kroon and Frank Berendse



Nardus stricta L.

Abstract

In this study we examined the effect of differences in root foraging ability on the population structure and the competitive ability of *Festuca rubra* and *Anthoxanthum odoratum* that were grown in monocultures and mixtures in homogeneous and heterogeneous environments during two growing seasons. The species have comparable growth rates but are known, from previous studies, to differ in root morphological and physiological plasticity, and in the ability to acquire nutrients from heterogeneous environments. Nutrient heterogeneity was introduced at two spatial scales, coarse- and fine-grained. The nutrient-rich patches in the fine-grained heterogeneous treatment were smaller and more concentrated than the nutrient-rich patches in the coarse-grained heterogeneous treatment, but the overall level of nutrient availability in these heterogeneous treatments was equal to the overall nutrient availability in the homogeneous treatment.

The results of the experiment clearly showed that nutrient heterogeneity affected the population structure and the competitive ability of the species. Size inequality in the populations of both species was significantly higher in the fine-grained heterogeneous treatment than in the homogeneous and coarse-grained heterogeneous treatment. The competitive ability of the species was estimated from the replacement design methodology based on shoot biomass production. The competitive ability of *F. rubra* was significantly higher than that of *A. odoratum* in the homogeneous treatment during the experiment. However, in the heterogeneous environments the competitive ability of *F. rubra* declined relative to *A. odoratum*, resulting in the absence of significant differences between the competitive ability of the species in the heterogeneous treatments at the end of the experiment.

The degree of selective root placement of the species in the nutrient-rich and nutrient-poor patches in the heterogeneous treatments, as determined by the root biomass produced within the patches in the monocultures, did not differ at the end of the experiment. In the coarse-grained heterogeneous treatment, no difference in root biomass between the nutrient-rich and nutrient-poor patches could be detected, neither for *F. rubra* nor for *A. odoratum*, but in the fine-grained heterogeneous treatment, both species were able to produce significantly more root biomass in the nutrient-rich patches than in the nutrient-poor patches.

The nutrient acquisition ability of the species was assessed by determining the amount of strontium captured by a species at the end of the experiment. $SrCl_2$ was injected in nutrient-

rich patches in the heterogeneous treatments and in a coinciding location in the homogeneous treatment three weeks prior to the end of the experiment. *F. rubra* tended to acquire more Sr in the homogeneous treatments, but in the coarse-grained heterogeneous treatment *A. odoratum* tended to acquire more Sr than *F. rubra,* though both not significantly so. These observations led us to conclude that *A. odoratum* has foraging mechanisms that result in a higher competitive ability in heterogeneous environments relative to *F. rubra.*

Keywords: competition, heterogeneity, nutrients, replacement design, root foraging, size inequality, nutrient tracers

Introduction

Nutrient heterogeneity is ubiquitous within natural habitats (Jackson and Caldwell 1993; Gross et al., 1995; Ryel et al., 1996; Cain et al., 1999). Plants have frequently shown to be able to proliferate roots and to enhance root uptake kinetics in response to patchily available nutrients, resulting in the acquisition of adequate amounts of nutrients in heterogeneous environments (Hutchings & De Kroon, 1994; Robinson, 1994; Robinson and Van Vuuren, 1998). The degree of root proliferation in response to nutrient heterogeneity is species specific (Crick & Grime, 1987: Jackson & Caldwell, 1989; Caldwell et al., 1991; Gross et al., 1993; Fransen et al., 1998), as well as nutrient specific (Drew, 1975; Jackson and Caldwell, 1989).

The ecological significance of root proliferation, particularly in response to N-enriched patches, has been obscure for a long time. Species display similar degrees of root proliferation in response to NO_3^--enrichment as to PO_4^--enrichment, even though the high mobility of NO_3^- limits the contribution of root proliferation to N capture (Robinson, 1996). Furthermore, the benefits of root proliferation in terms of nitrogen acquisition in heterogeneous environments are limited if patch depletion occurs (Fransen et al., 1998). Remarkably, root proliferation in response to N-enrichment may occur even after most of the N has been taken up (Van Vuuren et al., 1996). As a solution to these unexplained responses, it has recently been suggested that strong root proliferation in N-enriched patches may confer a selective advantage during interspecific competition for finite, local N-rich patches (Robinson et al., 1999).

Competition for nutrients in homogeneous environments is assumed to be relative size-symmetric, i.e. plants acquire nutrients in proportion to their biomass (Weiner, 1990; Casper and Jackson, 1997; Schwinning and Weiner, 1998; Berntson and Wayne, 1999). In heterogeneous habitats, however, larger plants may reach nutrient-rich patches and deplete nutrients before smaller plants can gain access, resulting in a disproportional acquisition of nutrients and asymmetric competition (Casper and Jackson, 1997; Weiner et al., 1997; Schwinning and Weiner, 1998). Hence, the patchy distribution of nutrients may affect the competitive ability of plants even if the total nutrient availability of the habitat is invariant.

Root plasticity differences between species can be expected to either increase or decrease size inequality within populations. Root plasticity differences will increase size inequality by increasing the differences in nutrient uptake and growth between the individuals of different species if species are roughly the same size, but differ in their ability to alter root morphology or physiology in response to nutrient heterogeneity. Root plasticity will also increase size inequality if the species are of different size and if the larger species is more plastic than the smaller species, resulting in more readily access to nutrient-rich patches. In contrast, root plasticity will reduce size inequality by reducing the differences in resource uptake and growth between large and small individuals if the smaller species is more plastic than the larger species. It has been suggested that, large, dominant plant species tend to maximise nutrient uptake by monopolising large volumes of soil through the production of extensive root systems. This 'high scale' foraging response contrasts the 'high precision' foraging response of small, subordinate plant species that tend to exploit small patches of high nutrient availability (Campbell et al., 1991).

The purpose of this study was to examine whether a higher ability to forage for patchily distributed resources confers a competitive advantage in heterogeneous environments. The effects of nutrient heterogeneity and competition on individuals and populations are examined using two perennial grass species i.e. *Anthoxanthum odoratum* L. and *Festuca rubra* L. The two species co-occur in natural grasslands and have comparable growth rates, but differ in root foraging ability and nutrient acquisition ability in homogeneous and heterogeneous environments (Fransen et al., 1998, 1999). The two species are both able to increase root length density in response to nutrient enrichment, but the increment in root length density was only significant for *Festuca rubra* (Fransen et al., 1998). However, the root morphological response of *Festuca rubra* resulted only in a small, non-

significant increase in nitrogen acquisition in a heterogeneous treatment compared to a homogeneous treatment (Fransen et al., 1998). In contrast, *Anthoxanthum odoratum* acquired significantly more nitrogen in the heterogeneous environment than in the homogeneous environment, probaly due to root physiological plasticity (Fransen et al., 1999). Hence, based on these previous results we predict that the competitive ability of *Anthoxanthum odoratum* will increase in heterogeneous environments relative to homogeneous environments. However, it is still unknown whether a high degree of root physiological plasticity can confer a competitive advantage in heterogeneous environments, or that such advantages can only be achieved by rapid root proliferation, due to a high degree of root morphological plasticity (cf. Robinson et al., 1999).

Here we describe an experiment, in which the species were grown for two years in monocultures and mixtures in homogeneous and heterogeneous environments. Nutrient heterogeneity was introduced at two spatial scales. The overall nutrient availability in these heterogeneous treatments was equal to that in the homogeneous treatment. The size of individual plants of both species did not significantly differ at the start of the experiment. Plant-level measurements included the root biomass production in the nutrient-rich and nutrient-poor patches in the monocultures. The application of a $SrCl_2$-solution to nutrient-rich patches enabled the determination of the nutrient acquisition ability by each of the species in the monocultures and the mixtures. Population-level measurements included mean shoot biomass, mean aboveground biomass, plant size inequality and competitive ability. Competitive ability was assessed using replacement designs (De Wit, 1960). The following hypotheses are tested:

1) The degree of selective root placement in response to nutrient heterogeneity does not differ between *Festuca rubra* and *Anthoxanthum odoratum*.
2) *Anthoxanthum odoratum* will acquire relatively more nutrients in the heterogeneous environments compared to the homogeneous environments than *Festuca rubra*, due to its higher degree of root physiological plasticity.
3) The competitive ability of *Anthoxanthum odoratum* relative to *Festuca rubra* will increase in the heterogeneous environments compared to the homogeneous environments, due to the acquisition of more nutrients in the heterogeneous environments by *Anthoxanthum odoratum*.

Materials and methods

Species

In the experiment two grass species, *Festuca rubra* L. and *Anthoxanthum odoratum* L. were grown for two growing seasons in monocultures and mixtures on both homogeneous and heterogeneous environments. The two grass species are characteristic of moderately nutrient-poor habitats. Potential relative growth rate of *Festuca rubra* is 1.18 $week^{-1}$ and of *Anthoxanthum odoratum* 0.94 $week^{-1}$ (Grime and Hunt, 1975).

The original plants of the two species were collected at various sites that differed in nutrient availability within a former agricultural grassland along the Anloeër Diepje, a brook in the 'Drentse Aa' Nature Reserve (53°N, 6°40'E) (see Bakker, 1989). The plants used in this study are propagated from the field material in a heated greenhouse with supplemental lighting from high-pressure sodium lamps (Philips SON-T 400W) giving a photoperiod of 12h. At the start of the experiment, young tillers, isolated from four original plants, of each species were randomly assigned to an initial harvest or to the experiment.

Experimental design

The experiment was carried out in an open greenhouse. In May 1997, large containers (90×70×40 cm) were either filled with a homogeneous soil, a coarse-grained heterogeneous soil or a fine-grained heterogeneous soil and contained 30 individuals (i.e. 48 individuals/m^2, Fig. 1). Individual tillers of each species were planted in a regular pattern in a standard replacement design, i.e. the monocultures of the two species and their 0.5:0.5 mixture. Each treatment×planting combination was replicated 6 times.

PVC-frames were placed in each container before filling to maintain the same soil compaction in each treatment. At the bottom of each container a layer of 5cm gravel was placed and covered with root cloth, to ensure drainage.

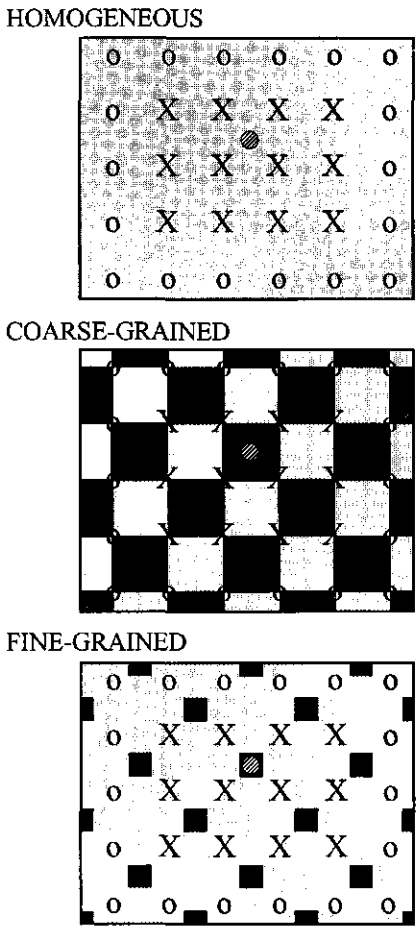


Figure 1. The planting pattern and the three soil treatments used in the experiment. The nutrient-rich patches in the heterogeneous treatments, indicated by darker areas, differed in size and nutrient concentration. The planting locations are indicated by O and X. The plants consisted either of 100% of each species in the monocultures or 50% of each species in the mixtures. In the mixtures, the planting positions of the two species were alternated. The plants indicated with X are included in the analysis, the rest is used to reduce edge effects. The hatched circle indicates the position of the $SrCl_2$ injection in each treatment.

The different treatments were constructed by using different mixture of humus-rich black soil and coarse sand. The total amount of the humus-rich black soil and sand used in each treatment was equal. Hence, the overall nutrient availability in each treatment was equal.

In the homogeneous treatment, the frame consisted of 20 cells of 15×15cm that were filled with a homogeneous soil mixture consisting of 12.5% black soil and 87.5% sand (mineral-N = 2.72mgN/kg, 1M KCl-extraction).

In the coarse-grained heterogeneous treatment, the same frame was used, but the cells were filled with either a mixture of 20% black soil and 80% sand (the nutrient-rich patches; mineral-N = 4.02mgN/kg, 1M KCl-extraction) or with a mixture of 5% humus-rich black soil and 95% sand (i.e. nutrient-poor patches; mineral-N = 1.65mgN/kg, 1M KCl-extraction).

In the fine-grained heterogeneous treatment, a frame that consisted of 10 cells of 6×6 cm was used. These small cells were filled with 100% humus-rich black soil (the nutrient-rich patches; mineral-N = 8.67mgN/kg, 1M KCl-extraction), and were on exact the same positions as the nutrient-rich patches in the coarse-grained heterogeneous treatment. The rest of the container was filled with nutrient-poor soil, consisting of 5% humus-rich black soil and 95% sand (mineral-N = 1.65mgN/kg, 1M KCl-extraction). The nutrient-rich patches in the fine-grained heterogeneous treatment were smaller and more concentrated than the nutrient-rich patches in the coarse-grained heterogeneous treatment. The soil moisture content in each container was kept at ±10% (mass%) by weighing the containers once a week and watering them three times a week during the experiment.

At the end of both the first (i.e. October 1997) and the second (i.e. September 1998) growing seasons, plants were clipped at a height of 2cm and divided in living and dead leaves and flowering stalks if present. The species did not flower during the first growing season, but both species flowered massively during the second growing season. The harvested biomass was dried at 70°C for at least 48h prior to weighing. Biomass production, size inequality and competitive ability of the species were determined using only the 12 centre plants in each container to reduce possible edge effects (Fig. 1).

To determine the degree of selective root placement of the species, 6 soil-cores, equally divided of nutrient-rich and poor patches, were taken in the monocultures in each treatment at the end of the experiment. Roots in these soil-cores were washed clean of soil particles and root biomass in the soil-cores was determined after drying at 70°C for at least 48h. Soil-cores were also taken in the mixtures in each treatment, but the roots of the two species could not be distinguished with certainty.

Strontium injection and analysis

Three weeks prior to the end of the experiment, 15 ml 0.2M $SrCl_2*(6H_2O)$, containing 263mg strontium, was injected in a nutrient-rich patch in the heterogeneous treatments at a depth ranging from 5-20cm. In the homogeneous treatment, the same solution was injected on a position equivalent to the injected nutrient-rich patch in the heterogeneous treatments. Strontium (Sr^{2+}) is physiologically analogous to Ca^{2+}, and can be used as a tracer to assess the root activity of different coexisting plant species (Veresoglou and Fitter, 1984; Mamolos et al., 1995).

In this experiment, strontium uptake is measured and used as an estimation of the ability of the species to exploit the nutrient-rich patches in the heterogeneous treatments. The four plants surrounding the injected nutrient-rich patch are used to determine the Sr-acquisition of the species. Living shoot biomass of the four plants was dried at 70°C for at least 48h and ground prior to the analyses. To determine the amount Sr taken up by the individual plants, 0.5g of the ground material was dry-ashed in an oven at 500°C for at least 4h and the ash was dissolved in 10ml 2M HCl (Mamolos et al., 1995). The Sr-concentration in this solution was measured by atomic absorption spectrometry (SpectrAA 600, Varian, The Netherlands).

Replacement design

In a standard replacement design, first described by De Wit (1960), total plant density in the mixture is equal to the plant density used in the monoculture of each component species. The replacement design is extremely valuable for comparing the competitive ability of two plant species under different conditions (Berendse, 1981; Berendse, 1982; Firbank and Watkinson, 1990).

The competitive ability of a species is given by the relative crowding coefficient (k_{12}) of a species (De Wit, 1960). The relative crowding coefficient indicates the competitive ability of a species during inter-specific competition, relative to its competitive ability during intra-specific competition. It should be noted that the relative crowding coefficient is not an

inherent species characteristic, but depends on the experimental conditions and harvest time. The relative crowding coefficient is defined as (De Wit, 1960):

$$O_1=k_{12}z_1(k_{12}z_1+z_2)^{-1} M_1$$

$$O_2=k_{21}z_2(k_{21}z_2+z_1)^{-1} M_2$$

Where $O_{1,2}$ is the biomass produced by species 1 and 2 respectively in the mixture, $z_{1,2}$ indicates the initial plant frequencies of species 1 and 2 in the mixture ($z_1+z_2=1$) and $M_{1,2}$ is the biomass produced by species 1 and 2 respectively in the monoculture.

The validity of replacement designs to study competition has been under much debate the last decade (Connolly, 1986; Snaydon, 1991; Cousens, 1991;Cousens and O'Neill, 1993; Sackville Hamilton, 1994; Snaydon, 1994; Gibson et al., 1999). The two main points of criticism on replacement designs are that the result is size-biased, favouring initially larger species (Connolly, 1986; Grace et al., 1992; Connolly, 1997) and that the result may be density-dependent (Firbank and Watkinson, 1985; Connolly, 1986; Austin et al., 1988; Taylor and Aarssen, 1989; Snaydon, 1991). De Wit (1960) already pointed out that the competitive ability of species, indicated by relative crowding coefficient, could only be properly analysed at densities at which all resources are absorbed, i.e. at a constant final yield. Hence, the above mentioned density-dependence of replacement designs is restricted solely to densities below the value at which a constant final is reached.

To avoid misinterpretations in the assessment of the competitive ability of the species in our experiment, resulting from an incorrect use of the replacement design, we made sure that the initial plants of *F. rubra* and *A. odoratum* used in the experiment did not differ (P=0.21) in size. Individuals of *F. rubra* had an average (±SE) dry weight of 47.9±5.5 mg (n=22), individuals of *A. odoratum* weighted 40.3±3.3 mg (mean ±SE, n=38). To check whether constant final yield is reached in the treatments, we determined the yield-density curves for both species in an additional experiment. In this additional experiment plants were grown in a homogeneous environment in containers that had a smaller surface area ($0.22m^2$) but were of equal depth as the containers used in the main experiment. In the additional experiment, plants were grown in densities of 2, 4, 8, 12 and 24 individuals per container, corresponding with densities of 9, 18, 36, 54 and 108 individuals m^{-2}, respectively. The yield-density curves of the species, showed that both species had an asymptotic yield-density relationship and that both *F. rubra* and *A. odoratum* reached their constant final yield at the plant density (48 individuals m^{-2}) used in the competition-experiment (Fig. 2).

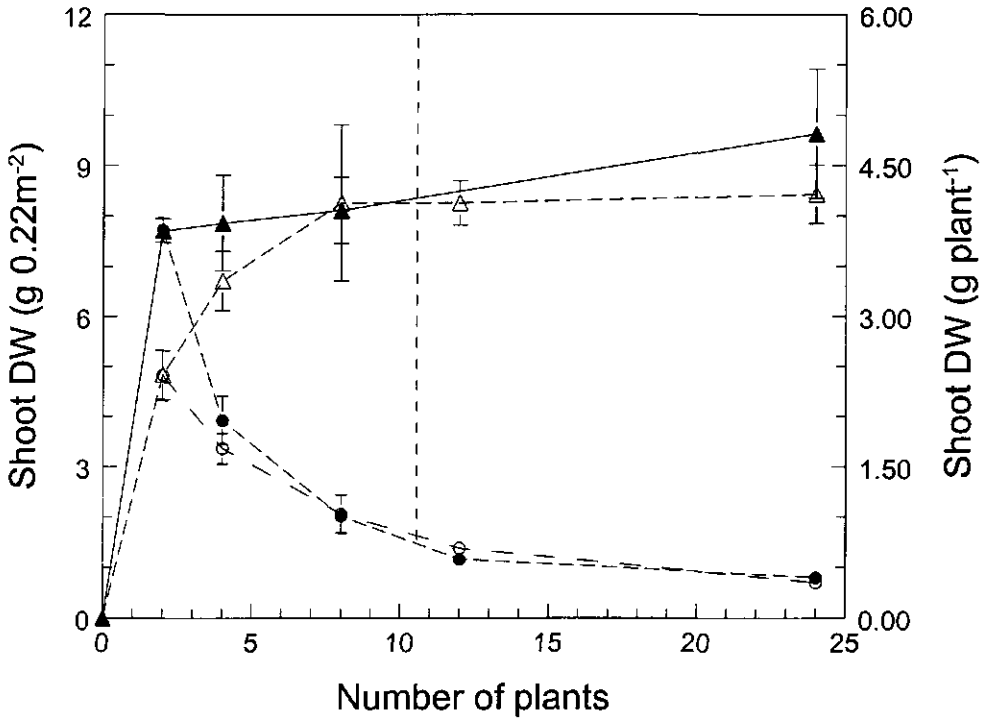


Figure 2. The yield density-curves (triangles) and the mean shoot biomass (circles) of *Festuca rubra* (solid markers) and *Anthoxanthum odoratum* (open markers) under a homogeneous nutrient distribution, as determined in an additional experiment. Data are means ± SE (n=4). The vertical, hatched line indicates the plant density used in the competition-experiment (i.e. 48 individuals/m^2).

Statistical analysis

Analysis of variance (GLM-procedure, SPSS 1995) was used to analyse root foraging ability, nutrient acquisition, biomass production and coefficients of variation in biomass production. Data were ln-transformed where necessary to ensure normality and homogeneity of variance. A posteriori comparisons were carried out using Tukey's-HSD test where appropriate.

Results

Selective root placement

The degree of selective root placement of the species in response to nutrient heterogeneity, as assessed by root biomass production within the patches, was similar (Table 1), even though *F. rubra* produced overall significantly ($P<0.05$) more root biomass than *A. odoratum*. Surprisingly, in the coarse-grained heterogeneous treatment neither species was able to produce significantly more root biomass in the nutrient-rich patches than in the nutrient-poor patches (Fig. 3B; $P>0.05$). In contrast, in the fine-grained heterogeneous treatment both species were able to selectively produce more root biomass in the nutrient-rich patches than in the nutrient-poor patches (Fig. 3C; $P<0.05$). Overall, the species produced significantly ($P<0.05$) less root biomass in the homogeneous and coarse-grained heterogeneous treatment than in the fine-grained heterogeneous treatment.

In the mixtures, no distinction could be made between roots produced by *F. rubra* and *A. odoratum*. However, the root biomass production in the mixtures followed exactly the same pattern in response to the different soil treatments as mentioned above. The species produced only significantly ($P<0.05$) more root biomass in the nutrient-rich patch than in the nutrient-poor patch in the fine-grained heterogeneous treatment (Fig. 3C; $P<0.05$)

Table 1. Analysis of variance, using a general linear model for the root biomass produced within the nutrient-poor and nutrient-rich patches in the different treatments. Groups in stead of species were used in the analyses, because in the mixtures no distinction could be made between roots produced by *F. rubra* and by *A. odoratum*. A group may consist either of *F. rubra* plants grown in monocultures, of *A. odoratum* plants grown in monocultures, or of plants of both species grown in mixtures. Data were ln-transformed prior to analysis. F-values are given.

Effects	df	Root biomass
Group	2	30.59***
Treatment	2	16.35***
Nutrient patch within treatment	3	17.09***
G×N within T	10	0.43 ns
Error	90	

ns=not significant, *P<0.05, **P<0.01, ***P<0.001

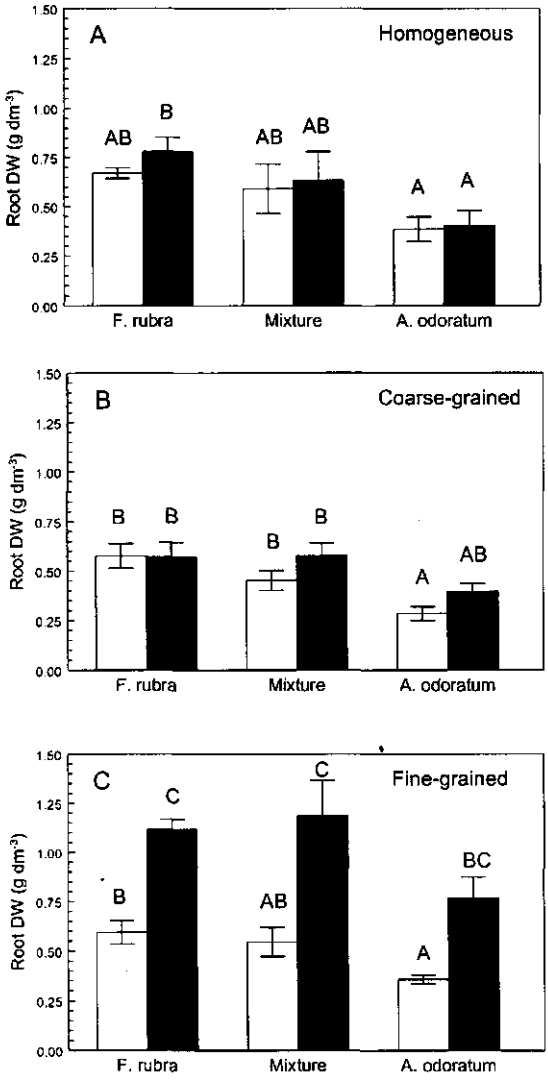


Figure 3. Root biomass production in the nutrient-rich (solid bars) and nutrient-poor (open bars) patches of the species in monocultures and mixtures in the homogeneous (A), the coarse-grained heterogeneous (B) and the fine-grained heterogeneous treatment (C), based on 3 soil cores taken in each patch type. Note, the nutrient-poor and nutrient-rich patches in the homogeneous treatment refer to their equivalent positions in the heterogeneous treatments, naturally no differences in nutrient concentration exists between the nutrient-rich and poor patches in this treatment. Data are means ± SE (n=6) Bars with the same letter are not significantly different (Tukey-HSD, P>0.05).

Nutrient acquisition ability

The nutrient acquisition ability of the species was assessed by the amount of strontium (Sr^{2+}) acquired from the $SrCl_2$-injected nutrient-rich patches (Fig. 4). Overall, the amount of Sr taken up in monocultures did not significantly (P>0.05) differ between *F. rubra* and *A. odoratum*, nor between the different treatments (Table 2).

Table 2. Analysis of variance, using a general linear model for the total amount of Sr acquired in the different treatments at the end of the second growing season. Data were ln-transformed prior to analysis. F-values are given.

Effects	df	Sr
Species	1	0.60 ns
Treatment	2	2.17 ns
Competition	1	4.84*
Species×Treatment	2	0.26 ns
Species×Competition	1	0.68 ns
Treatment×Competition	2	1.34 ns
Species×Treatment×Competition	2	1.08 ns

ns=not significant, *P<0.05, **P<0.01, ***P<0.001

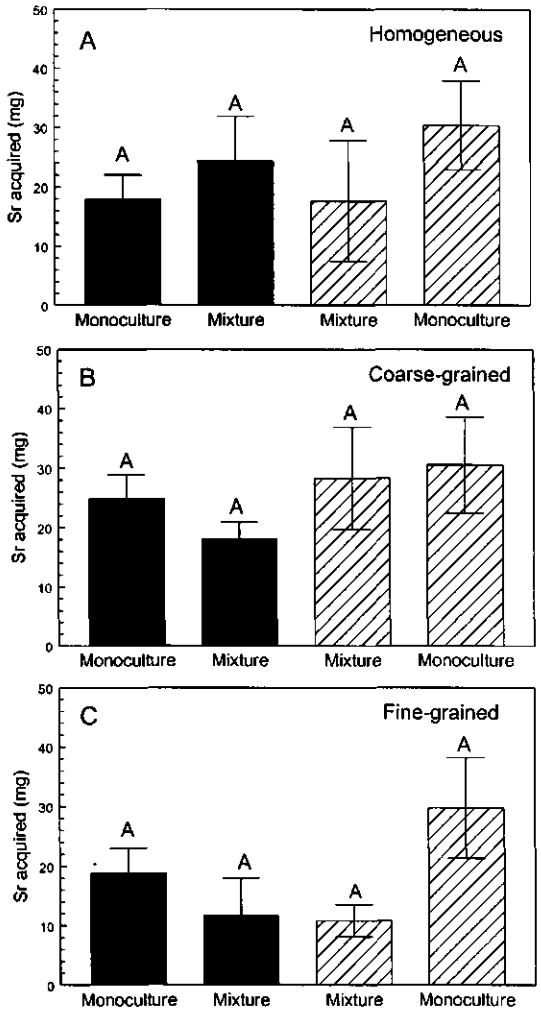


Figure 4. The average amount of Sr acquired per plant by *Festuca ru*bra (solid bars) and *Anthoxanthum* odoratum (hatched bars) grown in monocultures and mixtures in the mixtures in the homogeneous (A), the coarse-grained heterogeneous (B) and fine-grained heterogeneous treatment (C). Note: In the monocultures, the total amount of Sr is based on the acquisition of Sr by four plants; in the mixtures, the amount is based on the acquisition of Sr by two plants. Data are means ± SE (n=6) Bars with the same letter are not significantly different (Tukey-HSD, P>0.05).

The species acquired overall significantly more Sr in the monocultures than in the mixtures (Table 2). The amount of Sr acquired in the mixtures did again not significantly ($P>0.05$) differ between *F. rubra* and *A. odoratum*, nor between the treatments. However, *F. rubra* tended to acquire the highest amount of Sr in the homogeneous treatment (Fig. 4A) whereas *A. odoratum* tended to acquire the highest amount of Sr in the coarse-grained heterogeneous treatment (Fig. 4B), but the species×treatment interaction was not significant (Table 2).

Biomass production and plant size inequality

The mean shoot biomass per individual during the experiment (Fig. 5) did not differ between the species, but differed significantly between the treatments and the two growing seasons (Table 3). Overall, mean shoot biomass of the species was significantly ($P<0.05$) higher in the homogeneous and coarse-grained heterogeneous treatments than in the fine-grained heterogeneous treatment. Mean shoot biomass production per individual was also significantly higher in the second year than in the first year ($P<0.05$).

In the monocultures, mean shoot biomass production per individual of *A.odoratum* was overall significantly higher than of that of individuals of *F. rubra* in the first year ($P<0.05$), but not in the second year ($P>0.05$). Similarly, mean shoot biomass of the species was significantly higher in the homogeneous and coarse-grained heterogeneous treatment than in the fine-grained heterogeneous treatment at the end of the first year ($P<0.05$), but this difference had disappeared at the end of the second year ($P>0.05$).

In the mixtures, mean shoot biomass of individuals of *F. rubra* was overall significantly higher than mean shoot biomass of *A.odoratum*, both in the first and in the second year ($P<0.05$). Mean shoot biomass of the species was again significantly higher in the homogeneous and coarse-grained heterogeneous treatment than in the fine-grained heterogeneous treatment at the end of the first year ($P<0.05$), but this difference had also disappeared at the end of the second year ($P>0.05$).

It is noteworthy that the species responded differently to the form of competition (i.e. intra- vs. interspecific) as indicated by the significant species×competition interaction (Table 3). This significant interaction is mainly due to the significant ($P<0.05$) lower mean shoot

biomass per individual of *A. odoratum* in the mixtures than in the monocultures in the homogeneous and coarse-grained heterogeneous treatments at the end of the first growing season (Fig 5A,B).

In the second year, mean total aboveground biomass production per individual (including shoot- and flower stalk biomass production) was also analysed, because the species flowered massively during this growing season. In the monocultures, mean total aboveground biomass was overall significantly ($P<0.05$) higher for *A. odoratum* than for *F. rubra.* In the mixtures, although overall no significant differences ($P>0.05$) in mean total aboveground biomass between the species could be detected, *F. rubra* produced significantly ($P<0.05$) more aboveground biomass than *A. odoratum* in the homogeneous treatment, but this difference was absent in the heterogeneous treatments (Fig. 5).

Table 3. Analysis of variance, using a general linear model for mean shoot biomass produced in the first and second growing season (Shoot DW), for mean aboveground biomass in the second growing season (Above DW) and for their accompanying coefficients of variation (CV). Aboveground biomass includes shoot and flower stalk biomass. Biomass data were ln-transformed prior to analysis. F-values are given.

Effects	df	Shoot DW	CV	df	Above DW	CV
Species	1	1.19 ns	4.04*	1	0.01 ns	0.00 ns
Treatment	2	23.78***	27.94***	2	1.21 ns	13.59***
Competition	1	1.45 ns	8.80**	1	4.26*	8.46**
Year	1	48.87***	17.88***	--	--	--
S×T	2	1.87 ns	1.09 ns	2	4.57*	4.04*
S×C	1	27.82***	24.44***	1	13.03**	16.28***
S×Y	1	0.59 ns	0.22 ns	--	--	--
T×C	2	0.02 ns	0.39 ns	2	2.53 ns	1.32 ns
T×Y	2	5.43**	0.20 ns	--	--	--
C×Y	1	0.01 ns	0.01 ns	--	--	--
S×T×C	2	2.90 ns	6.91**	2	1.66 ns	5.01*
S×T×Y	2	1.75 ns	0.56 ns	--	--	--
S×C×Y	1	2.35 ns	0.42 ns	--	--	--
T×C×Y	2	0.91 ns	0.04 ns	--	--	--
S×T×C×Y	2	0.85 ns	0.49 ns	--	--	--
Error	120			60		

ns=not significant, *P<0.05, **P<0.01, ***P<0.001

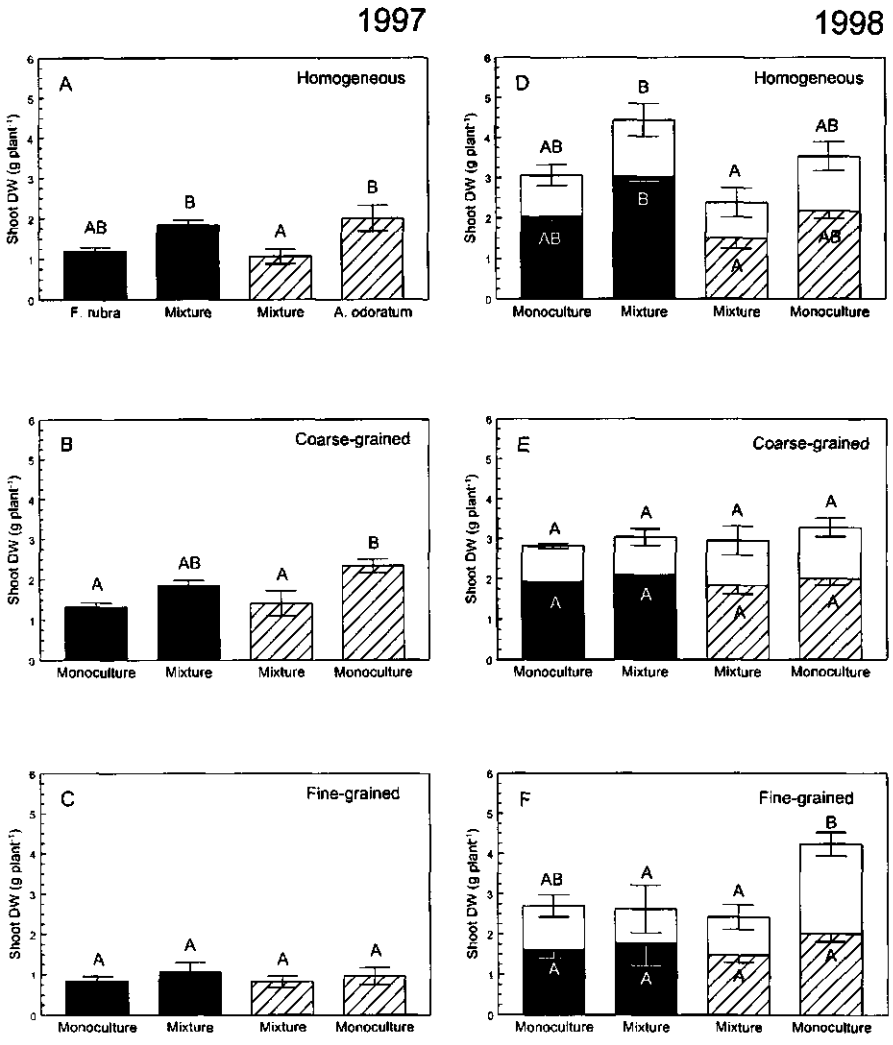


Figure 5. The average shoot biomass per plant of *Festuca rubra* (solid bars) and *Anthoxanthum odoratum* (hatched bars) in the homogeneous, the coarse-grained heterogeneous and the fine-grained heterogeneous treatment in the first (1997) and the second (1998) growing season. The stacked bars in the second growing season (1998) indicate the mean total aboveground biomass (including shoot and flower stalk biomass) production. Note: the data are based on the 12 centre plants in each treatment. Data are means ± SE (n=6) Bars with the same letter are not significantly different (Tukey-HSD, P>0.05).

Size inequality was assessed using the coefficient of variation (CV) in individual shoot biomass (Fig. 6). In the monocultures, the CV of *A. odoratum* was significantly ($P<0.05$) higher than the CV of *F. rubra*, both in the first growing season as in the second growing season. The CV in individual shoot biomass of the species was, in both years, significantly ($P<0.05$) higher in the fine-grained heterogeneous treatment than in the homogeneous and coarse-grained heterogeneous treatments.

In the mixtures, the CV did not significantly ($P>0.05$) differ between *F. rubra* and *A. odoratum*, neither in the first, nor in the second year. The CV in individual shoot biomass of the species in the mixtures was significantly ($P<0.05$) higher in the fine-grained treatment than in the coarse-grained heterogeneous treatment in the first year. In the second year, the CV in individual shoot biomass of the species in the fine-grained heterogeneous treatment was also significantly ($P<0.05$) higher than in the homogeneous treatment.

Remarkably, the CV of the species in response to the form of competition (i.e. intra- vs. interspecific) was treatment dependent as indicated by the significant species×treatment×competition interactions (Table 3). The CV of *F. rubra* and *A. odoratum* did not differ significantly between the monocultures and the mixtures in the homogeneous treatment. In the heterogeneous treatments, however, the CV of *F. rubra* tended to increase in the mixtures when compared to the monocultures, but the CV of *A. odoratum* decreased significantly in the mixtures when compared to the monocultures, except in the fine-grained heterogeneous treatment at the end of the first-growing season (Fig. 6).

The CV in total aboveground biomass per individual of the species in the second year displayed exactly the same pattern as the CV in individual shoot biomass. (results not shown).

Analysis of competitive ability

The yield-curves of *F. rubra* and *A. odoratum* in the different treatments in the two growing seasons, based on total shoot biomass production, are shown in Figure 7. In the homogeneous treatment, *F. rubra* shows a concave curve in both years, indicating that total shoot biomass production of *F. rubra* in the mixtures is higher than expected based on the yield in the monocultures. *A. odoratum* shows a convex curve in the homogeneous treatments in both years, indicating that the yield in the mixtures is lower than expected based on the yield in the

monocultures. The competitive ability of *F. rubra* and *A. odoratum* was assessed by the relative crowding coefficient of the species (k_{ij}, de Wit, 1960), based on the relative shoot biomass production of the species, in both growing season. In the homogeneous treatment the competitive ability of *F. rubra* was significantly higher than the competitive ability of *A. odoratum* in both the first and the second year (Fig. 7; Table 4).

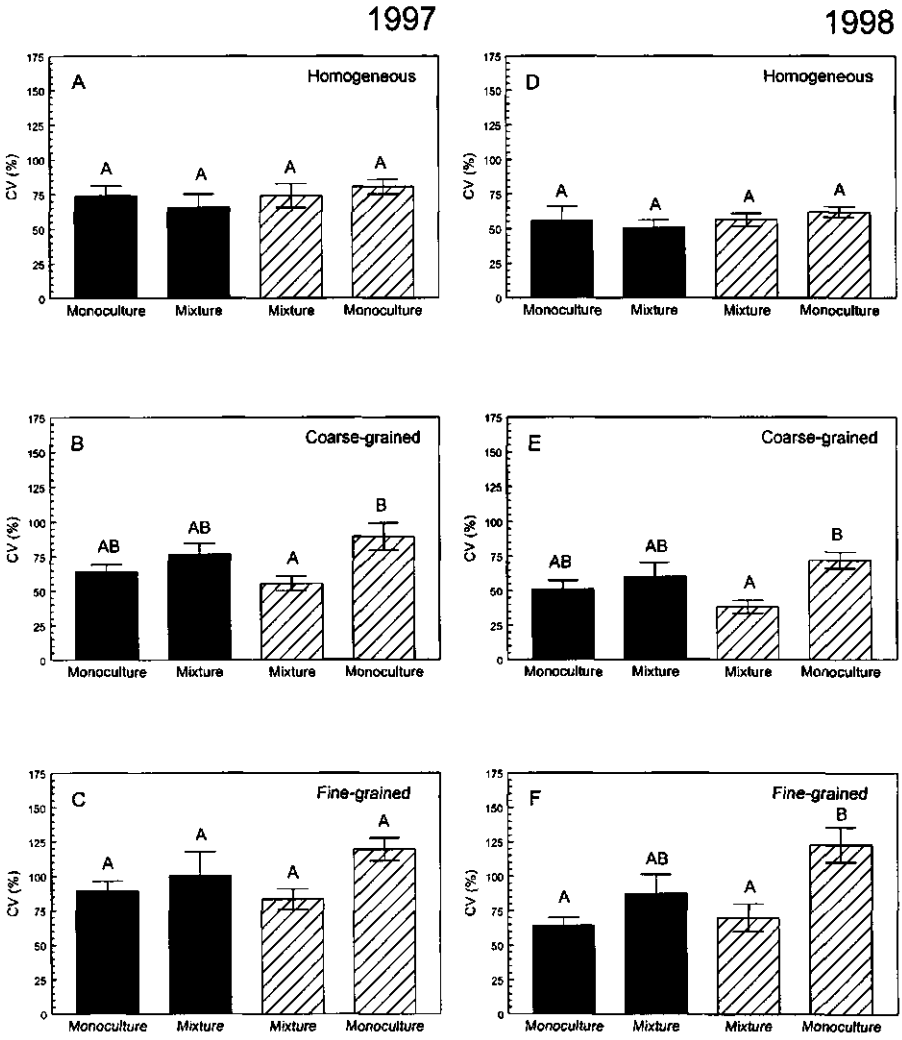


Figure 6. The coefficient of variation in shoot biomass of *Festuca rubra* (solid bars) and *Anthoxanthum odoratum* (hatched bars) in the homogeneous, the coarse-grained heterogeneous and the fine-grained heterogeneous treatment in the first (1997) and the second (1998) growing season. Note: the data are based on the 12 centre plants in each treatment. Data are means ± SE (n=6) Bars with the same letter are not significantly different (Tukey-HSD, P>0.05).

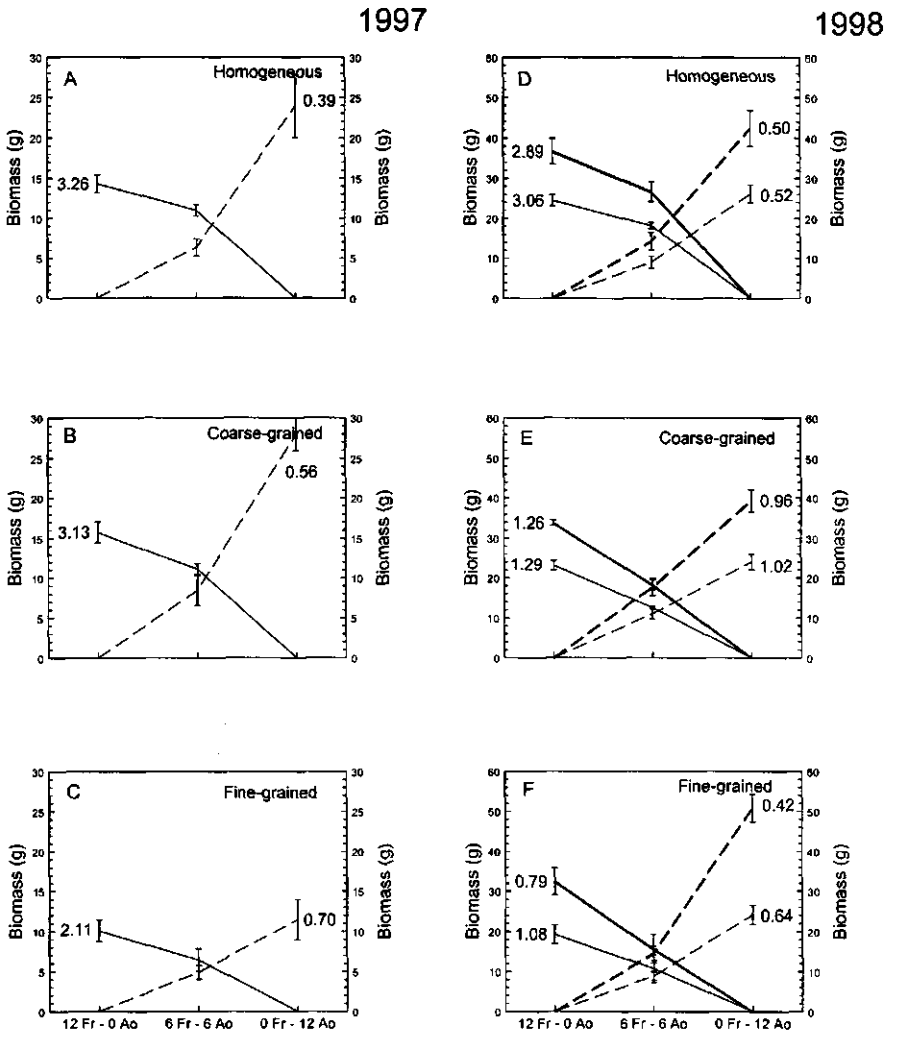


Figure 7. The yield density-curves based on the shoot biomass production of *Festuca rubra* (thin solid lines) and *Anthoxanthum odoratum* (thin broken lines) in the homogeneous, the coarse-grained heterogeneous and the fine-grained heterogeneous treatment in the first (1997) and second growing season (1998). The thick lines in the second growing season (1998) indicate the yield density-curves of the species based on the total aboveground biomass (including shoot and flower stalk biomass) production. The numbers represent the average values of the relative crowding coefficients (k; De Wit, 1960) of each species. On the x-axis, the number of plants of each species present is indicated. Note: the data are based on the 12 centre plants in each treatment.

In the heterogeneous treatments, *F. rubra* still shows a concave curve and *A. odoratum* shows a convex curve, but the curves of both species become more linear, particularly in the second growing season, indicating that the competitive ability of the species alters in the heterogeneous treatments compared to the homogeneous treatment. In the coarse-grained heterogeneous treatment the competitive ability of *F. rubra* is, in the first year, significantly higher than that of *A. odoratum*, but this difference disappears in the second growing season. In the fine-grained heterogeneous treatment, the competitive abilities of *F. rubra* and *A. odoratum* do not significantly differ during the two growing seasons (Table 4). Hence, the competitive ability of *A. odoratum* relative to *F. rubra* increases in the coarse-grained heterogeneous treatment compared to the homogeneous treatment, but this shift is less obvious in the fine-grained heterogeneous treatment.

The yield-curves of *F. rubra* and *A. odoratum* based on total aboveground biomass production in the different treatments in the second growing seasons are also shown in Figure 7D-F. The yield-curves based on the total aboveground biomass are, in general, identical to the yield-curves based on the total shoot biomass. The competitive ability of *F. rubra* based on the total aboveground biomass is significantly higher the competitive ability of *A. odoratum* in the homogeneous treatment, but in the heterogeneous treatments the competitive abilities of both species do not significantly differ (Table 4.)

Table 4. Relative crowding coefficients (k) of *Festuca rubra* and *Anthoxanthum odoratum* in the different nutrient-distribution treatments based on the total shoot biomass in the first (1997) and the second year (1998). In the second year, k based on the total aboveground biomass (i.e. shoot biomass and flower stalk biomass) is also given (1998 Total). Data are means±SE (n=4-6). Data were ln-transformed prior to analysis. Means with the same superscript are not significantly different within columns (Tukey-HSD (P=0.05) after one-way ANOVA).

		1997	1998	1998 Total
Festuca rubra	Homogeneous	3.26 ± 0.63^{b}	3.06 ± 0.65^{b}	2.89 ± 0.77^{c}
	Coarse-grained	3.13 ± 0.75^{b}	1.29 ± 0.18^{a}	1.26 ± 0.21^{bc}
	Fine-grained	2.11 ± 1.29^{ab}	1.08 ± 0.45^{a}	0.79 ± 0.25^{ab}
Anthoxanthum odoratum	Homogeneous	0.39 ± 0.06^{a}	0.52 ± 0.08^{a}	0.50 ± 0.05^{ab}
	Coarse-grained	0.56 ± 0.24^{a}	1.02 ± 0.26^{a}	0.96 ± 0.24^{ab}
	Fine-grained	0.70 ± 0.17^{a}	0.64 ± 0.12^{a}	0.42 ± 0.07^{a}

Discussion

The purpose of this 2-year study was to examine whether differences in root foraging ability between species would invoke differences in competitive ability in heterogeneous compared to homogeneous environments. In the experiment *F. rubra* and *A. odoratum* were used, two perennial grass species that co-occur in natural habitats, have comparable growth rates, but that have different foraging strategies and different abilities to acquire nutrients from heterogeneous environments (Fransen et al., 1989; 1999). The results of our experiment clearly show that nutrient heterogeneity affected the competitive ability of species, as assessed by the relative crowding coefficient (De Wit, 1960), even though the total amount of nutrients available in the different treatments was the same. In the homogeneous treatment, the competitive ability of *F. rubra* was significantly higher than that of *A. odoratum*, but in the heterogeneous treatments, no significant differences in competitive ability could be detected between *F. rubra* and *A. odoratum* at the end of the experiment. Hence, in heterogeneous environments the competitive ability of *F. rubra* declined relative to *A. odoratum*.

Do these shifts in the competitive ability of the species in heterogeneous environments compared to homogeneous environments, result from differences between the species in nutrient acquisition ability in heterogeneous environments? The ability to produce selectively more root biomass in the nutrient-rich patches than in the nutrient-poor patches did not differ between the species. Both species produced significantly more biomass in the nutrient-rich patch than in the nutrient-poor patch only in the fine-grained heterogeneous treatment after two years. However, although the degree of selective root placement of the species did not differ, the amount of root biomass produced overall within the soil-cores differed significantly between the species. Overall, *F. rubra* produced significantly more root biomass than *A. odoratum*, indicating that *F. rubra* has a more extended root system than *A. odoratum* which enables the exploitation of larger volumes of soil and results in a higher competitive ability in homogeneous soils (cf. Weiner, 1990; Casper and Jackson, 1997; Schwinning and Weiner, 1998; Berntson and Wayne, 1999).

In homogeneous environments, nutrient uptake is assumed to be relative size-symmetric, i.e. plants acquire nutrients in proportion to their biomass (Weiner, 1990; Casper and Jackson, 1997; Schwinning and Weiner, 1998). When competition is symmetric, it will not exacerbate initial size differences (Weiner et al., 1997). If we use the CV's in individual

shoot biomass of both species in the monocultures in the homogeneous treatment as a measure of the initial size differences and compare them to the CV's in the mixtures, no increment in CV can be detected, suggesting that competition for nutrients was indeed relative-size symmetric in the homogeneous treatment in the experiment.

The ability of the species to acquire nutrients in the homogeneous and heterogeneous treatments was assessed by quantifying the amount of strontium captured by the species from an $SrCl_2$-injected patch. Although the amount of Sr acquired in the mixtures did not differ significantly between the species in none of the treatments, *F. rubra* tended to acquire more Sr than *A. odoratum* in the mixtures in the homogeneous treatment. In contrast, in the coarse-grained heterogeneous treatment *A. odoratum* tended to acquire more Sr than *F. rubra*, suggesting that *A. odoratum* is better able to acquire nutrients in this heterogeneous environment than *F. rubra*. The amount of nutrients that a species can acquire when grown in competition may determine the competitive ability of species that is based on the biomass production of the species. However, the implicit assumption that resource acquisition is directly proportional to subsequent growth has not been tested for plants growing in competition (Berntson and Wayne, 1999). Our results at least suggest that nutrient acquisition of a species in mixtures relative to monocultures is indicative of the competitive ability of the species.

In heterogeneous environments, larger plants may reach nutrient-rich patches and deplete nutrients before smaller plants can gain access, resulting in a disproportional acquisition of nutrients and asymmetric competition (Weiner et al., 1997; Schwinning and Weiner, 1998). However, differences in root plasticity between species may counteract this increment in size inequality, particularly if the smaller species is able to monopolise the nutrient patches and obtains a disproportionate share of the nutrients, a process referred to as negative asymmetry (Weiner et al., 1997). In the experiment, *A. odoratum* indeed tended to acquire an disproportionate share of the available nutrients in the heterogeneous environments, indicated by the significant decline in CV of *A. odoratum* in the mixtures compared to the monocultures. The significant decline in CV of *A.odoratum* and the increase, though not significant, in the CV of *F. rubra* in the mixtures relative to the monocultures suggests that competition was indeed negatively asymmetric in the heterogeneous soils.

We propose that the decline of the competitive ability of *F. rubra* relative to *A. odoratum* is due to the fact that *A. odoratum*, in spite of the smaller size of the root system, is

able to reach and deplete the nutrient-rich patches before *F. rubra* gains access. The depletion of the nutrient-rich patches in the heterogeneous treatments by *A. odoratum* is probably due to its higher root physiological plasticity (see Fransen et al., 1998; 1999), because the degree of selective root placement of the species in the monocultures did not differ. However, we cannot be absolutely sure that *A. odoratum* exploited the patches due to a higher degree of root physiological plasticity, because the degree of selective root placement of both species may have changed in the mixtures. Root morphological plasticity of plants may be altered markedly when grown in competition (Caldwell, 1994; Huber-Sannwald et al., 1996). The presence of roots of other species may alter root morphology (Jastrow and Miller, 1993) and root elongation rate (Mahall and Callaway, 1992; Krannitz and Caldwell, 1995), resulting in a tendency for roots to segregate, i.e. to avoid each other, within nutrient-rich patches (Caldwell et al., 1991; Caldwell et al., 1996).

In conclusion, the significantly higher competitive ability of *F. rubra* relative to *A. odoratum* in the homogeneous treatment declined in the heterogeneous treatments. The observed shift in the competitive balance between the species in the heterogeneous treatments did not result from differences in selective root placement between the species. Other foraging characteristics seem to play a prominent role, e.g. root physiological plasticity. Hence, nutrient heterogeneity and root foraging ability may have a profound impact on the competitive balance of species in natural habitats.

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Summary

Spatial and temporal nutrient heterogeneity are ubiquitous in natural habitats, at scales relevant to individual plants. Plants have developed root foraging mechanisms to acquire adequate amounts of nutrients in these heterogeneous environments. Foraging in plants is accomplished by morphological changes in response to environmental conditions, and may result in the selective placement of roots within nutrient-rich patches. The ability of plants to proliferate roots in nutrient-rich patches due to alterations in root morphology is shown frequently, but both the timing and the degree of root proliferation varies widely between species. Species from inherently nutrient-rich habitats display in general a higher relative increase in root density in nutrient-rich patches than species from inherently nutrient-poor habitats. This observation prompted the hypothesis that root foraging mechanisms differ between species from habitats of different nutrient availability.

Hence, this thesis focuses on the question: Do species from habitats that differ in nutrient availability utilise different foraging mechanisms to acquire heterogeneously distributed soil resources, and do these foraging characteristics contribute to the success of the species in their indigenous habitats? To answer this question, the root foraging ability and its consequences for the nutrient acquisition of five perennial grass species that are characteristic of habitats ranging from inherently nutrient-rich to nutrient-poor, i.e. *Lolium perenne* L., *Holcus lanatus* L., *Festuca rubra* L., *Anthoxanthum odoratum* L. and *Nardus stricta* L. respectively was determined. Five species from a single family (Gramineae) were deliberately used in the analyses to avoid confounding effects of gross differences in growth form between species of different families and phylogeny.

It is important to make an unambiguous distinction between foraging and growth. Foraging precedes and enhances resource uptake whereas growth follows from resource uptake. However, a major problem exists with distinguishing foraging from growth, because some of the plastic responses that accomplish the foraging ability cannot be achieved without growth. The observed higher relative increase in root density in nutrient-rich patches of fast-growing species from inherently nutrient-rich habitats compared to slow-growing species from inherently nutrient-poor habitats may result from differences in growth rate rather than from differences in foraging ability. In chapter 2, a model is developed to disentangle the effects of foraging and growth rate on the selective root placement of species. It is shown theoretically that the observed difference in the ability to selectively place roots in

heterogeneous environments between species from nutrient-rich habitats and nutrient-poor habitats may result from differences in growth rate between the species rather than from differences in morphological plasticity. The model shows that when analysed at a common time, as is done in most empirical studies, fast-growing species from nutrient-rich habitats will produce relatively more roots in nutrient-rich patches, and obtain relatively more nutrients from these patches, than slow-growing species from inherently nutrient-poor habitats, even though their degree of morphological plasticity is equal.

In a short-term experiment (chapter 3), the root morphological and physiological characteristics of two species from nutrient-rich (*Lolium perenne* and *Holcus lanatus*) and two species from nutrient-poor habitats (*Festuca rubra* and *Anthoxanthum odoratum*) in response to spatial and temporal nutrient heterogeneity were examined. Nutrient heterogeneity was created by injecting equal amounts of nutrient solution into the soil either on one location (i.e. creating spatial heterogeneity) or on several, alternating locations (i.e. creating temporal heterogeneity). On regular times ^{15}N-enriched nutrient solution was injected. To determine the consequences of the root morphological and physiological responses for the nutrient acquisition of the species, the amount of nitrogen captured in the heterogeneous treatments is compared with the amount captured in a homogeneous treatment in which the same amount of nutrient solution was spread homogeneously over the soil surface. After 27 days, all species produced significantly more root biomass within the nutrient-enriched patch in the spatial heterogeneous treatment. This increment in root biomass occurred without significant alterations in specific root length, branching frequency or mean lateral root length, but probably resulted from a local increase in the number of main root axes. The degree of the increment in root biomass was similar for species from nutrient-rich habitats and species from nutrient-poor habitats. In response to the temporal heterogeneous treatment, all species increased the ^{15}N-uptake rate per unit root biomass. Although the species from nutrient-poor habitats had, on average, higher uptake rates per unit root biomass than species from nutrient-rich habitats, the degree of the increment, i.e. root physiological plasticity did not significantly differ between the species. These short-term results question the validity of the assumption that root foraging characteristics differ among species form nutrient-rich and nutrient-poor habitats. As a result of their root responses, all species, irrespective of their indigenous habitat, captured significantly more nitrogen in the spatial- and temporal heterogeneous treatment than in the homogeneous treatment.

A longer-term experiment (2-3 months) with five perennial grass species ranging from inherently nutrient-rich to inherently nutrient-poor habitats (i.e. *Lolium perenne, Holcus*

lanatus, *Festuca rubra*, *Anthoxanthum odoratum* and *Nardus stricta*, respectively) was carried out (chapter 4) to assess the importance of root morphological responses for the capture of nutrients from soil. In natural soils, patch depletion occurs: a gradual decline in the nutrient supply of patches due to nutrient uptake and leaching. Patch depletion may reduce the benefits of selective root placement. In this experiment heterogeneity was created by placing soil mixtures that differed in nutrient availability in different spatial arrangements. To determine the ability of the species to acquire nutrients from spatial heterogeneous environments, a heterogeneous treatment was compared with a homogeneous treatment that had the same overall nutrient availability. All species, on average, increased their root length density in response to nutrient-enrichment as a result of small insignificant increases in root biomass and specific root length, but the increment was only significant for the faster-growing species from the nutrient-richer habitats. Overall, the species acquired significantly more nitrogen in the heterogeneous treatment than in the homogeneous treatment. However, the ability to acquire nutrients (nitrogen or phosphorus) in the heterogeneous treatment was not related to the ability of the species to increase root length density in response to local nutrient enrichment. For example, *Anthoxanthum odoratum*, a species that did not significantly place more roots selectively in the nutrient-enriched patch, acquired significantly more nitrogen in the heterogeneous than in the homogeneous treatments due to root physiological plasticity. These results indicate that the profits of root proliferation in terms of nutrient acquisition for species are limited when patch depletion occurs, and suggest that root physiological plasticity, which enables nutrient uptake prior to patch depletion, may be more important for the acquisition of nutrients in heterogeneous habitats.

In addition to the effects of patch depletion, the occurrence of root turnover may further limit the profits of root proliferation in long-term experiments. The merits of root foraging are often defined in terms of nutrient-uptake or short-term growth, but the long-term growth of perennial plants in natural habitats is dependent on the balance between nutrient uptake and nutrient losses. To include the effect of root turnover and the concomitant nutrient losses on the net long-term nutrient acquisition, *Holcus lanatus*, a species characteristic of nutrient-rich habitats, and *Nardus stricta*, a species characteristic of inherently nutrient-poor habitats, were grown individually in heterogeneous and homogeneous nutrient-rich and nutrient-poor environments under two overall levels of nutrient availability (chapter 5). Since root mortality will primarily occur during winter, the experiment lasted for two growing seasons. The plants were grown in under two overall levels of nutrient availability, but the relative difference between the nutrient-rich and nutrient-poor patch in the heterogeneous

treatment was the same under both overall levels of nutrient availability. The same contrast may invoke equal root foraging responses, but the benefits of root foraging may be lower in the overall nutrient-poor environments, because plants may not be able to acquire sufficient nutrients to offset their nutrient losses due to root turnover. Surprisingly, *Holcus lanatus* the species that was able to selectively place more roots in the nutrient-rich side in the heterogeneous treatment tended to produce less biomass in the heterogeneous treatment than expected based on its biomass production in the homogeneous treatments under both overall levels of nutrient availability. In contrast, *Nardus stricta* did not display selective root placement, but was able to produce the expected amount of biomass in the heterogeneous treatments under both overall levels of nutrient availability. Remarkably, the difference in relative shoot biomass production between *Holcus lanatus* and *Nardus stricta* in the heterogeneous treatment did not result from differences in root turnover rates between the species. Root turnover rates were estimated from minirhizotron observations. The differential response in relative shoot biomass production was probably due to the production of more roots in the nutrient-rich side of the heterogeneous treatment by *Holcus lanatus* than necessary to acquire all available nutrients. Hence, root proliferation may in the long-term even be disadvantageous in terms of biomass production instead of advantageous as suggested by the short-term experiments.

If root proliferation does not confer an ecological advantage in natural habitats and may eventually even result in a selective disadvantage, then why is this response so widespread among plants? The answer may lie in the competitive environment in which plants grow in natural habitats. The ability of a plant to proliferate roots in nutrient-enriched patches may be critical to its belowground competitive success. Hence, the costs of overproducing roots in nutrient-rich patches may outweigh the risk of other plants gaining advantage in terms of nutrient acquisition.

To determine the effects of differences in root foraging ability and nutrient acquisition on the competitive ability of species in heterogeneous environments, *Festuca rubra* and *Anthoxanthum odoratum* were grown in monocultures and mixtures in homogeneous and heterogeneous environments during two growing seasons (chapter 6). These species had shown to differ in the ability to acquire nutrients from heterogeneous environments in former experiments (chapters 3, 4), probably due to differences in root physiological plasticity. Nutrient heterogeneity was introduced at two spatial scales, i.e. coarse- and fine-grained. The nutrient-rich patches in the fine-grained heterogeneous treatment were smaller and more concentrated than the nutrient-rich patches in the coarse-grained (checkerboard)

heterogeneous treatment. The overall nutrient availability in these heterogeneous treatments was equal to the overall nutrient level in the homogeneous treatment. The root foraging ability of the species was determined by comparing the root biomass production in the nutrient-rich patches with the root biomass in the nutrient-poor patches in the monocultures, at the end of the experiment. The ability of the species to acquire nutrients in the different treatments was determined by quantifying the amount of strontium (Sr) captured from patches in which $SrCl_2$ was injected. The competitive ability of the species was estimated from replacement design methodology based on the shoot biomass production of the species at the end of the first- and second growing season.

The results of this experiment clearly showed that nutrient heterogeneity affected the competitive ability of the species, even if the total amount of nutrients is invariant. *Festuca rubra* had a significantly higher competitive ability in the homogeneous treatment than *Anthoxanthum odoratum*, but the competitive ability of *F. rubra* declined relative to *A. odoratum* in the heterogeneous treatments. In the coarse-grained heterogeneous treatment, *A. odoratum* tended to acquire more, though not significantly so, nutrients than *F. rubra*, even though the degree of selective root placement of the species did not differ at the end of the experiment. These observations led us to conclude that the degree of root morphological plasticity in response to nutrient enrichment is less important for the competitive ability of species in heterogeneous environments than the speed with which a species can exploit nutrient-rich patches.

Overall, the results described in this thesis contradict the hypothesis that species from nutrient-rich habitats display a higher degree of root morphological plasticity than species from nutrient-poor habitats. The higher degree of selective root placement of species from more nutrient-rich habitats compared to species from more nutrient-poor habitats may result from differences in growth rate rather than from differences in root morphological plasticity. The results further indicate that the ability to selectively place more roots in nutrient-rich patches than in nutrient-poor patches may confer an advantage in terms of nutrient acquisition in heterogeneous environments in the short-term, but in the long-term the increased root density may result in a lower rather than a higher biomass production in heterogeneous environments. However, root foraging abilities by which local nutrient patches are exploited may still be profitable when plants are grown in competition. The ability to rapidly exploit nutrient-rich patches due to root foraging characteristics seems to confer a competitive advantage in heterogeneous environments, even in the long-term.

Samenvatting

Planten worden in hun natuurlijke standplaats geconfronteerd met een ruimtelijk en temporeel heterogeen aanbod van voedingsstoffen (nutriënten). Planten hebben mechanismen ontwikkeld die hen in staat stellen om voldoende nutriënten te verwerven in deze heterogene omgeving. Planten foerageren door de morfologie van hun wortelstelsel zodanig aan de omgevingscondities aan te passen (morfologische plasticiteit) dat deze veranderingen resulteren in selectieve plaatsing van wortels in nutriëntrijke plekken in de bodem. Het vermogen van planten om een uitgebreid wortelstelsel te produceren in nutriëntrijke plekken als gevolg van veranderingen in wortelmorfologie is veelvuldig aangetoond, maar zowel de snelheid als de mate van uitbreiding vertonen grote verschillen tussen plantensoorten. Soorten van nutriëntrijke standplaatsen vertonen in het algemeen een hogere relatieve toename in worteldichtheid in nutriëntrijke plekken dan soorten van nutriëntarme standplaatsen. Op basis van deze waarnemingen is de hypothese geformuleerd dat foerageermechanismen verschillen tussen soorten van standplaatsen die verschillen in nutriëntenrijkdom.

Om deze hypothese te toetsen is het foerageergedrag van wortels en haar gevolgen voor de verwerving van nutriënten van vijf meerjarige grassoorten, te weten *Lolium perenne* L. (Engels raaigras), *Holcus lanatus* L. (Gestreepte witbol), *Festuca rubra* L. (Rood zwenkgras), *Anthoxanthum odoratum* L. (Gewoon reukgras) en *Nardus stricta* L. (Borstelgras), bepaald. Deze grassoorten zijn karakteristiek voor standplaatsen die respectievelijk variëren van zeer nutriëntenrijk tot zeer nutriëntenarm. Om in de analyses effecten van uitgesproken verschillen in groeivorm en fylogenie (verwantschap) tussen soorten van verschillende families te vermijden, zijn met opzet vijf soorten van één familie (Gramineae) gebruikt.

Het is noodzakelijk om een ondubbelzinnig onderscheid te maken tussen foerageergedrag en groei. Foerageren gaat vooraf aan en vergroot de opname van nutriënten terwijl groei het gevolg is van de opname van nutriënten. Er bestaat echter een groot probleem om foerageergedrag te onderscheiden van groei. Sommige van de morfologische veranderingen die nodig zijn om te foerageren kunnen niet plaats vinden zonder groei. De waargenomen hogere relatieve toename in worteldichtheid in nutriëntrijke plekken door snelgroeiende soorten van nutriëntrijke standplaatsen vergeleken met langzaamgroeiende soorten van nutriëntarme standplaatsen hoeven niet het resultaat te zijn van verschillen in foerageermechanismen maar kunnen voortkomen uit verschillen in groeisnelheid. In

hoofdstuk 2 is een model ontwikkeld om de effecten van foerageren en groeisnelheid op de selectieve plaatsing van wortels in nutriëntrijke plekken te ontrafelen. De resultaten van het model tonen aan dat de waargenomen verschillen in het vermogen om selectief wortels te plaatsen in heterogene milieus tussen soorten van nutriëntrijke en nutriëntarme standplaatsen het resultaat kunnen zijn van verschillen in groeisnelheid en niet hoeven voort te komen uit verschillen in morfologische plasticiteit. Het model laat zien dat wanneer soorten vergeleken worden op één tijdstip, zoals vaak gedaan is in empirische studies, snelgroeiende soorten van nutriëntrijke standplaatsen relatief meer wortels produceren in nutriëntrijke plekken, en relatief meer nutriënten verwerven uit deze plekken, dan langzaamgroeiende soorten van nutriëntarme standplaatsen, zelfs bij een gelijke mate van morfologische plasticiteit.

In een kortlopend experiment (hoofdstuk 3) zijn de morfologische en fysiologische veranderingen in het wortelstelsel van twee soorten van nutriëntrijke (*Lolium perenne* en *Holcus lanatus*) en twee van nutriëntarme (*Festuca rubra* en *Anthoxanthum odoratum*) standplaatsen als reactie op een heterogeen aanbod van nutriënten bestudeerd. Heterogeniteit werd gecreëerd door gelijke hoeveelheden voedingsoplossing in de bodem te injecteren hetzij op één plek (creëren van ruimtelijke heterogeniteit) of op meerdere, afwisselende plekken (creëren van temporele heterogeniteit). Op gezette tijden werd voedingsoplossing die verrijkt was met gelabeld stikstof (^{15}N) geïnjecteerd. De gevolgen van morfologische en fysiologische veranderingen in het wortelstelsel van soorten voor de verwerving van nutriënten werden bepaald door de totale hoeveelheid stikstof die werd opgenomen door de soorten in de heterogene behandelingen te vergelijken met de hoeveelheid die werd opgenomen in een homogene behandeling waarin dezelfde hoeveelheid voedingsoplossing homogeen werd uitgespreid over de bodem. Na 27 dagen hadden alle soorten significant meer wortels geproduceerd in de nutriëntrijke plek in de ruimtelijk heterogene behandeling dan in de temporeel heterogene en homogene behandelingen. De relatieve toename in wortelbiomassa was gelijk voor soorten van nutriëntrijke en nutriëntarme standplaatsen. De toename in wortelbiomassa vond in alle soorten plaats zonder significante veranderingen in specifieke wortellengte, vertakkingsgraad of lengte van zijwortels. In de temporeel heterogene behandeling waren alle soorten in staat de ^{15}N opname snelheid per eenheid wortelbiomassa in de nutriëntrijke plekken te verhogen. De mate van toename in opname snelheid (fysiologische plasticiteit) verschilde niet significant tussen de soorten ook al hadden de soorten van nutriëntarme standplaatsen gemiddeld een hogere opname snelheid per eenheid wortelbiomassa dan soorten van nutriëntrijke standplaatsen. Deze resultaten zijn in tegenspraak met de hypothese dat de foerageer mechanismen van soorten uit nutriëntrijke en

nutriëntarme standplaatsen verschillen. Als gevolg van de morfologische en fysiologische veranderingen in hun wortelstelsel verwierven alle soorten, ongeacht de nutriëntenbeschikbaarheid hun natuurlijke standplaats, meer stikstof in de heterogene behandelingen dan in de homogene behandeling.

Een langer lopend experiment (2-3 maanden) werd uitgevoerd (hoofdstuk 4) om het belang van morfologische veranderingen in het wortelstelsel van soorten voor de verwerving van nutriënten uit natuurlijke bodems vast te stellen. In het experiment zijn vijf meerjarige grassoorten gebruikt die karakteristiek zijn voor standplaatsen die variëren van zeer nutriëntrijke tot zeer nutriëntarme, respectievelijk *Lolium perenne, Holcus lanatus, Festuca rubra, Anthoxanthum odoratum* en *Nardus stricta*. In natuurlijke bodems, treedt depletie van nutriëntrijke plekken op. Depletie is de geleidelijke uitputting van de nutriëntrijke plek als gevolg van de opname en uitspoeling. Depletie kan het voordeel van selectieve wortelplaatsing reduceren. In dit experiment werd heterogeniteit gecreëerd door bodemmengsels die verschillende hoeveelheden nutriënten bevatte, in verschillende ruimtelijke configuraties aan te bieden. Om het vermogen van de soorten om nutriënten te verwerven in ruimtelijk heterogene omgevingen te bepalen, werd de opname van stikstof en fosfaat in de heterogene behandeling vergeleken met de homogene behandeling die een zelfde totaal aanbod van nutriënten had. Gemiddeld genomen nam de wortellengtedichtheid van alle soorten toe als reactie op een lokale verrijking van nutriënten, maar de toename was alleen maar significant voor de snelgroeiende soorten van de nutriëntrijke standplaatsen. Deze toename in wortellengte dichtheid was in alle soorten het gevolg van kleine toenames in wortelbiomassa en specifieke wortellengte in deze nutriëntrijke plekken. De soorten verwierven meer stikstof in the heterogene behandeling dan in de homogene behandeling. Echter het vermogen van de soorten om nutriënten te verwerven in de heterogene behandeling was niet gerelateerd aan het vermogen om de wortellengtedichtheid te verhogen in de nutriëntrijke plek. Bijvoorbeeld, *Anthoxanthum odoratum*, een soort die niet in staat was significant meer wortels in de nutriëntrijke plek te plaatsen, verwierf, als gevolg van fysiologische plasticiteit, significant meer stikstof in de heterogene behandeling dan in de homogene behandeling. Deze resultaten geven aan dat het voordeel van selectieve wortelplaatsing in termen van nutriëntenopname beperkt is als depletie van de nutriëntrijke plekken optreedt en suggereren dat fysiologische plasticiteit, welke planten in staat stelt om nutriënten te verwerven voordat depletie optreedt, belangrijker kan zijn voor de verwerving van nutriënten in heterogene standplaatsen dan morfologische plasticiteit.

Een andere factor die naast depletie het voordeel van selectieve kan beperken wortelplaatsing op de lange termijn is het afsterven van wortels. De voordelen van selectieve wortelplaatsing worden vaak gedefinieerd in termen van nutriënt opname of korte termijn groei, maar de lange termijn groei van perenne soorten in hun natuurlijke standplaats is afhankelijk van de balans tussen de opname en de verliezen van nutriënten. Om de gevolgen van het afsterven van wortels en de daarmee gepaard gaande verliezen van nutriënten voor de lange termijn groei mee te nemen, werden *Holcus lanatus,* een soort karakteristiek voor nutriëntrijke standplaatsen en *Nardus stricta*, een soort karakteristiek voor nutriëntarme standplaatsen individueel opgekweekt in heterogene en homogeen nutriëntrijke en nutriëntarme groeiplaatsen (hoofdstuk 5). Het experiment liep gedurende twee groeiseizoenen, omdat het afsterven van wortels vooral optreedt in de wintermaanden. Het experiment werd uitgevoerd in twee series die een verschillend niveau van nutriënten beschikbaarheid hadden, maar het relatieve verschil (contrast) tussen de nutriëntrijke en nutriëntarme helft in de heterogene behandeling was identiek onder beide niveaus. Naar verwachting roept een zelfde contrast gelijke morfologische en fysiologische veranderingen in het wortelstelsel op, maar de voordelen van foerageermechanismen zullen lager zijn onder een laag niveau van nutriëntenbeschikbaarheid als planten niet in staat zijn om voldoende nutriënten te verwerven om het verlies van nutriënten als gevolg van het afsterven van wortels te compenseren.

Verrassend was dat *Holcus lanatus*, de soort die in staat was om selectief meer wortels te plaatsen in de nutriëntrijke helft van de heterogene behandeling onder beide niveaus van nutriëntenbeschikbaarheid, geneigd was om minder spruitbiomassa te produceren in de heterogene behandeling dan verwacht op basis van zijn spruitbiomassa productie in de homogene behandelingen. *Nardus stricta* daarentegen vertoonde geen selectieve wortelplaatsing, maar was in staat om de verwachte hoeveelheid spruitbiomassa in de heterogene behandeling te produceren onder beide algemene niveaus van nutriëntenbeschikbaarheid. Opmerkelijk was dat het verschil in relatieve spruitbiomassa productie in de heterogene behandeling tussen *Holcus lanatus* en *Nardus stricta* niet het gevolg was van verschillen in de snelheid waarmee de wortels van de beide soorten afsterven. De snelheid waarmee wortels afstierven werd bepaald aan de hand van minirhizotron opnames. Het verschil in relatieve spruitbiomassa productie is waarschijnlijk veroorzaakt doordat *Holcus lanatus* meer wortels geproduceerd heeft in de nutriëntrijke helft in de heterogene behandeling dan nodig om de aanwezige nutriënten te verwerven. Het lijkt er dus op dat selectieve wortelplaatsing op de lange termijn zelfs nadelig kan zijn in termen van

biomassa productie in plaats van voordelig zoals gesuggereerd wordt in kortlopende experimenten.

Als selectieve wortelplaatsing geen ecologisch voordeel oplevert in natuurlijke standplaatsen, en uiteindelijk zelfs een nadeel kan opleveren, waarom is deze respons dan zo wijd verbreid onder plantensoorten? Het antwoord kan liggen in het feit dat planten in hun natuurlijke standplaats onderling concurreren. Het vermogen om selectief wortels te plaatsen in nutriëntrijke plekken in de bodem kan van doorslaggevend belang zijn voor het succes van de soort in zijn natuurlijke standplaats. De nadelige gevolgen van een overproductie van wortels in nutriëntrijke plekken kunnen het risico compenseren dat andere planten voordeel verkrijgen in termen van nutriënten verwerving.

Om de effecten van verschillen in foerageergedrag en verwerving van nutriënten op het concurrentievermogen van soorten in heterogene omgevingen te bepalen werden *Festuca rubra* and *Anthoxanthum odoratum* opgekweekt in mono- en mengculturen in homogene en heterogene behandelingen gedurende twee groeiseizoenen (hoofdstuk 6). Uit eerdere experimenten (hoofdstuk 3 en 4) was gebleken dat deze soorten verschillen in het vermogen om nutriënten te verwerven in heterogene milieus, waarschijnlijk voornamelijk als gevolg van verschillen in fysiologische plasticiteit. Heterogeniteit werd aangeboden op twee ruimtelijke schalen, grof- en fijnschalig. De nutriëntrijke plekken in de fijnschalige heterogene behandeling waren kleiner en hadden een hogere concentratie aan nutriënten dan de nutriëntrijke plekken in de grofschalige heterogene behandeling (een schaakbord patroon). De totale hoeveelheid nutriënten in de heterogene behandelingen was gelijk aan het totaal aanbod van nutriënten in de homogene behandeling. Aan het eind van het experiment werd de wortelbiomassa die geproduceerd was in de nutriëntrijke plekken in de monoculturen in de verschillende behandelingen vergeleken met de wortelbiomassa die geproduceerd was in de nutriëntarme plekken. Het vermogen van de soorten om nutriënten te verwerven in de verschillende behandelingen werd bepaald door de hoeveelheid strontium (Sr^{2+}) die individuele planten hadden opgenomen uit met $SrCl_2$ verrijkte plekken te kwantificeren. Strontium is fysiologisch analoog aan calcium (Ca^{2+}). Het concurrentievermogen van de soorten werd geschat uit De Wit-vervangingsdiagrammen die gebaseerd waren op de spruitbiomassa van de soorten aan het einde van het eerste en tweede groeiseizoen.

De resultaten van dit experiment laten duidelijk zien dat heterogeniteit het concurrentievermogen van soorten beïnvloedt, zelfs als de totale hoeveelheid beschikbare nutriënten gelijk is. In de homogene behandeling was het concurrentievermogen van *Festuca rubra* significant groter dan dat van *Anthoxanthum odoratum*, maar in de heterogene

behandelingen nam het concurrentievermogen van *Festuca rubra* af ten opzichte van *Anthoxanthum odoratum*. In de grofschalige heterogene behandeling neigde *Anthoxanthum odoratum* meer nutriënten te verwerven dan *Festuca rubra*, zelfs terwijl de mate van selectieve wortelplaatsing gelijk was voor beide soorten. Deze resultaten leiden tot de conclusie dat de mate van veranderingen in wortelmorfologie als reactie op nutriëntrijke plekken minder belangrijk is voor het concurrentievermogen van soorten in heterogene omgevingen dan de snelheid waarmee een soort een verrijkte plek kan exploiteren.

In het algemeen spreken de resultaten beschreven in dit proefschrift de hypothese dat soorten van nutriëntrijke standplaatsen een grotere morfologische plasticiteit vertonen dan soorten van nutriëntarme standplaatsen tegen. De hogere mate van selectieve wortelplaatsing door soorten uit meer nutriëntrijke standplaatsen in vergelijking met soorten uit meer nutriëntarme standplaatsen kan het gevolg zijn van verschillen in groeisnelheid tussen de soorten en is niet noodzakelijk het resultaat van verschillen in morfologische plasticiteit. De resultaten geven verder aan dat het vermogen om selectief wortels te plaatsen in nutriëntrijke plekken in heterogene omgevingen op de korte termijn een voordeel kan opleveren met betrekking tot de verwerving van nutriënten, maar dat de toename in worteldichtheid op de lange termijn kan resulteren in een lagere in plaats van een hogere biomassa in heterogene omgevingen. Echter de foerageermechanismen waarmee lokale, nutriëntrijke plekken worden geëxploiteerd kan nog steeds een voordeel opleveren in een competitieve omgeving. Het vermogen om met behulp van foerageermechanismen snel nutriëntrijke plekken te exploiteren lijkt een competitief voordeel op te leveren in heterogene omgevingen, zelfs op de langere termijn.

Nawoord

Tijdens het doorbladeren van dit proefschrift is direct duidelijk dat dit proefschrift niet tot stand had kunnen komen zonder hulp van anderen. De hulp van collega's is vaak duidelijk, de hulp van vrienden is minder duidelijk en vaak is er een scheiding aanwezig tussen collega's en vrienden. Ik ben blij dat tijdens mijn promotieonderzoek het onderscheid tussen collega en vriend in een groot aantal gevallen is weggevallen, zonder dat dit geleid heeft tot eindeloos gediscussieer over werk in de vrije tijd.

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Bart Fransen
Zaltbommel, juli 1999

Curriculum vitae

Bart Fransen werd geboren op 31 maart 1971 te Eindhoven. In 1989 behaalde hij het Atheneum B diploma aan het Bisschoppelijk College te Weert. In september 1989 begon hij met de studie Biologie aan de Universiteit Utrecht. Tijdens zijn doctoraalfase voerde hij, in het kader van een hoofdvak Vegetatie-ecologie, een onderzoek uit naar fysiologische integratie binnen de klonale planten Ruige zegge en Zeegroene zegge. Voor het hoofdvak Internationale Natuurbescherming voerde hij, onder auspiciën van de Prins Bernard leerstoel voor Internationale Natuurbescherming, een onderzoek uit naar het ruimtelijke vegetatie-patroon van perenne grassen in savanne graslanden in Zimbabwe. In een bijvak Landschapsecologie beschreef hij effecten van vegetatiebeheer op klonale plantpopulaties in venen.

In juni 1995 studeerde hij af en aansluitend begon hij met zijn promotieonderzoek bij de leerstoelgroep Natuurbeheer en Plantenecologie van de Wageningen Universiteit. In deze periode onderzocht hij het foerageergedrag van planten wortels en de consequenties hiervan voor de verwerving van nutriënten en het concurrentievermogen van planten in heterogene omgevingen. De resultaten van dit onderzoek zijn vastgelegd in dit proefschrift.