

POLISH JOURNAL OF ECOLOGY (Pol. J. Ecol.)	55	4	731–746	2007
----------------------------------------------	----	---	---------	------

Regular research paper

Johann G. ZALLER

Institute of Organic Agriculture, University of Bonn, Germany.

(Present address): Institute of Zoology, University of Natural Resources and Applied Life Sciences
Vienna, Gregor Mendel Strasse 33, A-1180 Vienna, Austria, e-mail: johann.zaller@boku.ac.at

EFFECT OF PATCHY DISTRIBUTION OF SOIL NUTRIENTS ON ROOT MORPHOLOGY AND BIOMASS ALLOCATION OF SELECTED GRASSLAND SPECIES: EXPERIMENTAL APPROACH

ABSTRACT: Nutrient distribution in natural habitats is usually patchy in space and time, however most knowledge about plant growth and behaviour is based on experiments conducted under spatially homogenous conditions. Evidence has accumulated that the growth and competitive interaction of plant species are strongly affected by heterogeneous rather than homogeneous resource distribution, even when the total resource supply remains similar. For this study it is hypothesized that infestations of grasslands with the nitrophilous weed *Rumex obtusifolius* L. (broad-leaved dock) are partially the consequence of its ability to exploit spatial nutrient heterogeneity. This was tested in a full-factorial pot experiment with homogeneously or heterogeneously distributed nitrogen and/or potassium at either normal or increased soil moisture where *R. obtusifolius* was grown together with three other grassland species (grass: *Arrhenatherum elatius* L., non-leguminous herb: *Taraxacum officinale* Weber, leguminous herb: *Trifolium repens* L.). The species differed significantly in their root morphology (root length and diameter, specific root length, number of root tips) and biomass allocation response to nutrient distribution, as well as to the nutrient type used to create patches and to soil moisture (e.g., significant species \times treatment interactions). Generally, the root system of *A. elatius* showed the highest plasticity to imposed treatments, followed by *T. officinale*, *R. obtusifolius* and *T. repens*. Unexpectedly, root morphology of *R.*

obtusifolius was unresponsive to soil heterogeneity and less responsive to nutrient type and irrigation than that of the other species. Nutrient type used to create patches influenced the biomass allocation to the root system of *R. obtusifolius* while nutrient distribution and irrigation showed no effect on biomass allocation. Exploitation of soil nitrate-nitrogen and potassium was similar among species but exploitation was individually affected by nutrient type, nutrient distribution and irrigation suggesting that species-specific differences in nutrient storage capacities in roots or adjustments regarding root nutrient uptake kinetics may play an important role. Results from this study show that *R. obtusifolius* does not seem to have superior traits to utilize soil nutrient heterogeneity, certain nutrient types or higher soil moisture that differentiates it from the other grassland species tested. The observed effects might have consequences for the long-term competitive relationships between species in the grassland community suggesting that cultural and biological management measures oriented towards the improvement of the competitive ability of co-occurring grassland species might also be important in heterogeneous soils.

KEY WORDS: *Arrhenatherum elatius*, grassland, nutrient patches, resource use, *Rumex obtusifolius*, soil heterogeneity, *Taraxacum officinale*, *Trifolium repens*, weed ecology

1. INTRODUCTION

Although nutrient distribution in natural habitats is usually patchy in space and time (Jackson and Caldwell 1993, Gross *et al.* 1995, Ryel *et al.* 1996), most knowledge about plant growth and behaviour is based on experiments conducted under spatially homogenous conditions. Evidence has accumulated that growth of plant species is strongly affected by heterogeneous rather than homogeneous resource distribution, even when the total resource supply remains similar (Farley and Fitter 1999, Wijesinghe *et al.* 2001). Spatial heterogeneity in nutrients has been shown to affect placement and growth of leaves and roots, overall plant growth, the intensity of inter-plant competition and the yield and structure of plant populations and communities (Fransen *et al.* 2001, Facelli and Facelli 2002, Day *et al.* 2003a, Day *et al.* 2003b, Wijesinghe *et al.* 2005). Commonly, plants have developed foraging mechanisms that enable them to alter their root proliferation and morphology in response to nutrient enrichment (Jackson and Caldwell 1989, Gross *et al.* 1993, Pregitzer *et al.* 1993, Bilbrough and Caldwell 1995) and to increase their nutrient uptake rate per unit root biomass or length (Robinson and Rorison 1983, Jackson and Caldwell 1991, van Vuuren *et al.* 1996). Generally, soil resource heterogeneity is expected to be most beneficial to plants in soils of lower fertility (Reynolds and D'Antonio 1996).

As a result of management practices like fertilisation or irrigation, distribution of nutrients in agroecosystems is likely to be more homogenous than in more natural ecosystems. Nevertheless in pastures a high degree of soil nutrient heterogeneity can be expected given the fact that for instance cattle grazing at recommended stocking densities of two ruminants per hectare can create about 30 nutrient-rich patches within a single day by their defecating and urinating activities (J. Zaller, unpublished). *Rumex obtusifolius* is a troublesome weed in both grasslands (mainly pastures) and arable lands and an indicator for excessive soil nitrogen (e.g., Ellenberg 1986) or potassium concentrations (e.g. Kutschera and

Sobotik 1985). This species is particularly successful as a weed because of its ability to quickly establish from seeds (Cavers and Harper 1964), its high regenerative potential (Pino *et al.* 1995, Zaller 2004a, Zaller 2006b), possible allelopathic interference with competitors (Zaller 2006a) and because it can take up nitrate-nitrogen more efficiently than other plants (Rehder 1982). However, not much is known on how *R. obtusifolius* responds to nutrient patches and whether different levels of soil moisture can alter this response (Zaller 2004b). Generally, it is assumed that inherently fast-growing species 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). Therefore, this study tested the ability of *R. obtusifolius* as a fast-growing species and three other co-occurring perennial species (grass: *Arrhenatherum elatius*, non-leguminous herb: *Taraxacum officinale*, leguminous herb: *Trifolium repens*) to exploit and utilise soil patches enriched in nitrogen or potassium concentration at either normal or increased irrigation.

The specific objectives of this study were to test, whether 1) root morphology and biomass allocation of *R. obtusifolius* in response to soil nutrient heterogeneity, nutrient types and soil moisture levels differs from that of other grassland species and whether 2) species of differential functional groups with a different root system (i.e., tap root *vs.* adventitious root system) show specific response patterns. These species were chosen because they are early colonizers of sward gaps in grassland and often occur in the vicinity of *R. obtusifolius* and would thus be directly affected by *Rumex*' expansion in grasslands.

2. MATERIAL AND METHODS

2.1. Experimental site and plant material

The experiment was conducted between November 2002 and June 2003 in a greenhouse at the certified organic research farm of the University of Bonn, Germany.

Seed material of *R. obtusifolius* was collected at the research farm in October 2002, seeds of *A. elatius* (Poaceae), *T. officinale* (Asteraceae) and *T. repens* (Fabaceae) were obtained from a commercial supplier (Rieger-Hofmann, Blaufelden-Raboldshausen, Germany). All seeds were germinated in Petri-dishes on damp filter paper at controlled conditions (22°C, 12 hour day/night regime). When the first leaves developed after cotyledons the seedlings were planted into 4-L plastic pots (diameter 16 cm, height 20 cm) filled with a soil mixture (see below).

2.2. Creating soil nutrient heterogeneity and irrigation treatments

Patch fertilization treatments were used to create a heterogeneous soil environment in which roots of all four species had to compete for limited nutrient-rich areas. Pots were filled with homogenized and sieved (< 2 mm) sandy-loamy fluvisols obtained from the top 10 cm of an arable field of the research farm (C:N ratio = 11.3, P = 6.2 mg 100 g⁻¹, K = 21.4 mg 100 g⁻¹, soil bulk density: 1.4 g cm⁻³). Nutrient patches (diameter 2 cm, depth 10 cm) were created in the centre of each pot and were made of elastic nylon mesh material filled with soil enriched either with Kainite, a natural mineral salt that consists of potassium chloride and magnesium sulphate (K enrichment), horn powder (N enrichment) or both (K+N enrichment). For the treatment with homogeneously distributed nutrients, similar nutrient types and quantities were homogeneously mixed into the soil mixture used to fill the pots (see Table 1 for an overview of the nutrient concentrations in the established treatments). Nutrient levels

approximate conditions after long-term manure application (Zaller and Köpke 2004) or nutrient contents under decaying cow pats (Dickinson and Craig 1990).

One seedling per species was planted as seedlings in equidistance of 4 cm from the centre of the pots and had the same neighbouring species in all treatments. Because of this planting design, it was expected that roots of different species had an equal chance in finding and exploring nutrients distributed in patches. In order to find out how soil water content might affect plant responses to nutrient type and heterogeneity, two irrigation treatments had been established: for regular irrigation plants were watered with tap water when needed, for increased irrigation treatments plants received twice the amount of water at each watering date. Excessive water from increased irrigation was collected at the bottom of the pots; therefore a loss of nutrients from the pots could be prevented and nutrients could be reabsorbed by plants.

Each treatment was replicated seven times; pots with different treatments were randomly distributed on a greenhouse table and re-arranged once per week to minimise location effects. The experiment was established in November 2002 and lasted for 218 days; by the end of the experiment *T. officinale* started flowering.

2.3. Harvesting

Experimental pots were destructively harvested by vertically cutting the soil between plants into four similar sections. Plants growing in sections were then separated in above- and belowground parts to determine

Table 1. Initial nutrient concentrations in soil microsites enriched with potassium (K patch), nitrogen (N patch) or a combination of both (K+N patch) compared to surrounding soil in pots before planting. Means \pm SE, $n = 4$.

Sampling location	C %	N %	Available P (mg P kg ⁻¹)	Available K (mg K kg ⁻¹)
<i>K patch</i>	1.57 \pm 0.01	0.07 \pm 0.01	1.39 \pm 0.01	189.70 \pm 3.14
N patch	10.43 \pm 0.02	2.76 \pm 0.01	2.03 \pm 0.27	23.64 \pm 0.35
K+N patch	9.51 \pm 0.04	2.45 \pm 0.02	36.44 \pm 1.64	152.16 \pm 2.35
Surround. soil	2.40 \pm 0.04	0.16 \pm 0.00	2.44 \pm 0.27	10.29 \pm 1.1

Table 2. General effects of nutrient type (NT), nutrient distribution (ND), irrigation (I) and their interactions across species (Spp). Bold *P*-values indicate significant effects derived from ANOVAs.

Parameter	<i>P</i> -values of factors tested									
	Spp	NT	ND	I	Spp x NT	Spp x ND	Spp x I	NT x ND	NT x I	ND x I
Root length.	0.002	< 0.001	0.002	< 0.001	0.064	0.004	0.001	0.302	0.006	0.409
Root diameter	0.210	0.101	0.452	< 0.001	0.078	0.008	0.002	0.868	0.015	0.874
Specific root length	< 0.001	0.104	0.053	0.002	0.288	0.525	0.081	0.801	0.101	0.967
Root tips	< 0.001	0.028	0.221	< 0.001	0.041	0.050	0.004	0.389	0.424	0.513
Shoot mass	< 0.001	0.001	0.081	< 0.001	0.034	< 0.001	< 0.001	0.222	0.837	0.286
Root mass	< 0.001	0.002	0.031	< 0.001	0.485	0.059	< 0.001	0.767	0.157	0.104
Shoot/root ratio	< 0.001	0.016	0.662	0.005	0.001	0.893	0.004	0.794	0.424	0.780
Soil K content	0.212	< 0.001	< 0.001	< 0.001	0.002	0.330	0.666	0.057	0.118	0.034
Soil N content	0.322	< 0.001	< 0.001	< 0.001	0.805	0.650	0.064	0.074	0.245	0.022
Soil pH	0.954	0.072	0.101	< 0.001	0.583	0.454	0.463	0.301	0.166	0.301

Table 3. Root diameter (mm) of *Rumex obtusifolius*, *Arrhenatherum elatius*, *Taraxacum officinale*, and *Trifolium repens* that had been grown in soil with homogenous (homog.) and heterogeneous (heterog.) distribution of potassium (K), nitrogen (N) or both (K+N). Means \pm SE, n = 7. Abbreviations for ANOVA results: N–nutrient type (K, N, K+N), ND–nutrient distribution (homog., heterog.), I–irrigation (normal, increased). Bold *P*-values indicate significant effects.

Nutrient / Irrigation	<i>R. obtusifolius</i>		<i>A. elatius</i>		<i>T. officinale</i>		<i>T. repens</i>	
	homog.	heterog.	homog.	heterog.	homog.	heterog.	homog.	heterog.
Normal irrigation								
N fertil.	0.40 \pm 0.02	0.46 \pm 0.07	0.56 \pm 0.09	0.60 \pm 0.10	0.45 \pm 0.01	0.47 \pm 0.02	0.59 \pm 0.09	0.39 \pm 0.01
K fertil.	0.56 \pm 0.08	0.41 \pm 0.02	0.55 \pm 0.07	0.77 \pm 0.07	0.49 \pm 0.08	0.48 \pm 0.06	0.57 \pm 0.10	0.48 \pm 0.09
K+N fertil.	0.48 \pm 0.07	0.41 \pm 0.02	0.39 \pm 0.01	0.48 \pm 0.05	0.41 \pm 0.03	0.46 \pm 0.03	0.52 \pm 0.17	0.44 \pm 0.07
Increased irrigation								
N fertil.	0.41 \pm 0.01	0.40 \pm 0.01	0.42 \pm 0.01	0.43 \pm 0.01	0.47 \pm 0.02	0.50 \pm 0.08	0.41 \pm 0.00	0.38 \pm 0.01
K fertil.	0.39 \pm 0.01	0.46 \pm 0.06	0.41 \pm 0.02	0.39 \pm 0.01	0.50 \pm 0.06	0.41 \pm 0.01	0.41 \pm 0.01	0.37 \pm 0.01
K+N fertil.	0.52 \pm 0.08	0.44 \pm 0.07	0.42 \pm 0.01	0.40 \pm 0.02	0.40 \pm 0.02	0.40 \pm 0.02	0.47 \pm 0.07	0.40 \pm 0.01
ANOVA results	N	<i>P</i> = 0.376	N	<i>P</i> = 0.009	N	<i>P</i> = 0.043	N	<i>P</i> = 0.949
	ND	<i>P</i> = 0.227	ND	<i>P</i> = 0.071	ND	<i>P</i> = 0.591	ND	<i>P</i> = 0.025
	I	<i>P</i> = 0.622	I	<i>P</i> < 0.001	I	<i>P</i> = 0.907	I	<i>P</i> = 0.015
	N \times ND	<i>P</i> = 0.355	N \times ND	<i>P</i> = 0.505	N \times ND	<i>P</i> = 0.157	N \times ND	<i>P</i> = 0.791
	N \times I	<i>P</i> = 0.365	N \times I	<i>P</i> = 0.007	N \times I	<i>P</i> = 0.180	N \times I	<i>P</i> = 0.598
	ND \times I	<i>P</i> = 0.406	ND \times I	<i>P</i> = 0.032	ND \times I	<i>P</i> = 0.655	ND \times I	<i>P</i> = 0.282

allocation patterns. Only roots that could clearly be assigned to a plant species were used for further analyses while roots from unclear origin were discarded. Roots growing into the central nutrient patches were carefully excavated and included in the analyses of the particular plant species. Because experiments were conducted in an unheated greenhouse during winter/spring months with low temperatures above- and below-ground plant growth was rather low and therefore only little intermingling of roots occurred in the soil sections. The shoots of each plant species were dried at 60°C for 48 hours and weighed. Roots in soil sections were thoroughly washed free of attached soil using a 1-mm mesh screen, carefully spread

on a glass tray and digitized using a flatbed scanner. Root images were then analyzed for total root length, average root diameter and number of root tips using image analysis software (WinRhizo, Regent Instruments, Quebec, Canada). Each root sample was analysed three times, average measures were used for further data analysis. After measuring, roots were dried and weighed. As an indirect measure of root thickness, specific root length (SRL) was calculated as the root length per unit dry weight (m g^{-1}).

2.4. Soil nutrients

In order to determine exploitation of soil nutrients by plant species, at the harvest of

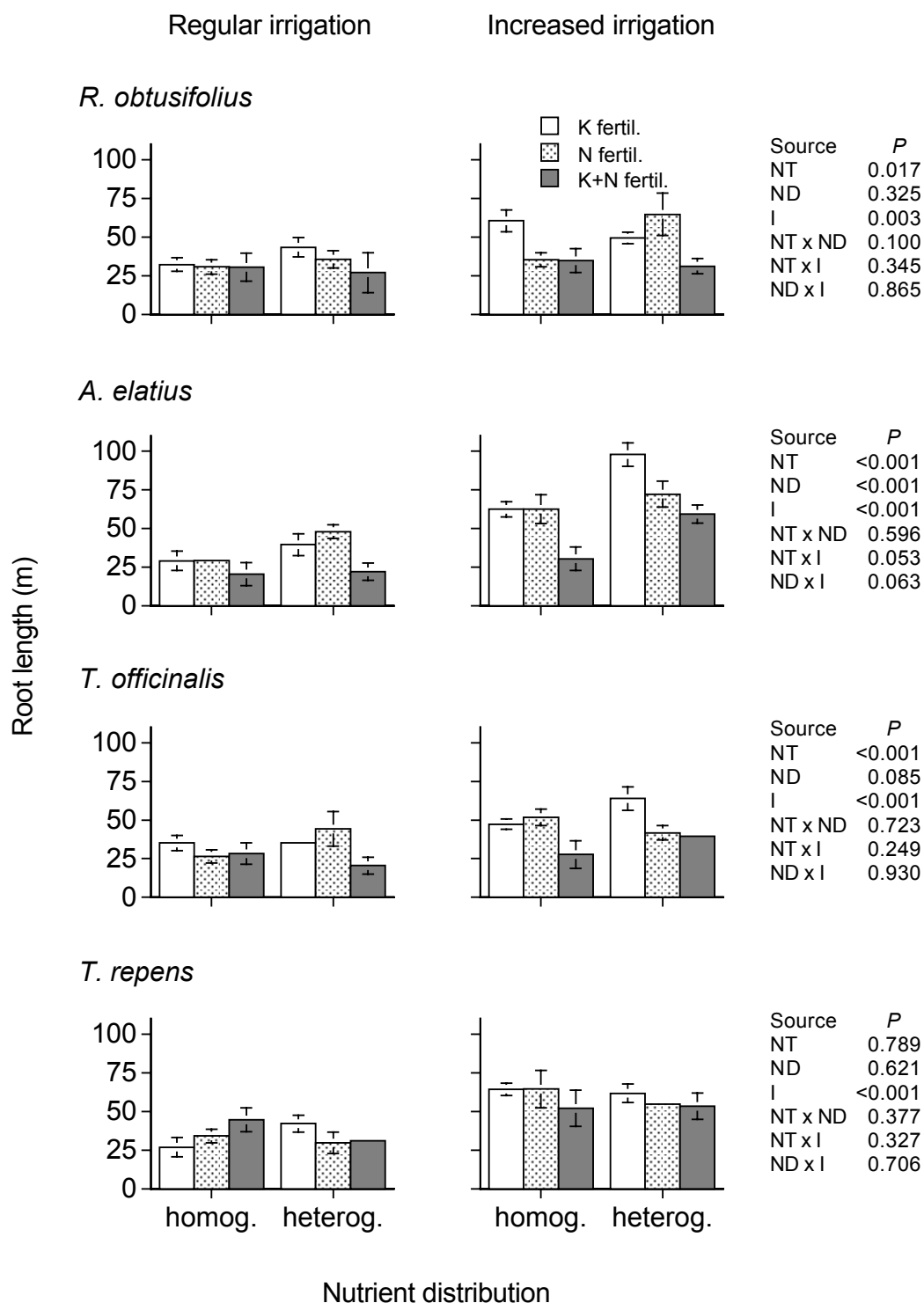


Fig. 1. Root length of grassland species grown in pots with either homogeneously or heterogeneously distributed nutrients (K, N, K+N) under regular or increased irrigation. Means \pm SE ($n = 7$). P values from ANOVA results with nutrient type (NT), nutrient distribution (ND) and irrigation (I) as factors. Small error bars are not depicted.

Table 4. Specific root length (m g^{-1}) of *Rumex obtusifolius*, *Arrhenatherum elatius*, *Taraxacum officinale*, and *Trifolium repens* that had been grown in soil with homogenous (homog.) and heterogeneous (heterog.) distribution of potassium (K), nitrogen (N) or both (K+N). Means \pm SE, $n = 6$. Abbreviations for ANOVA results: N–nutrient type (K, N, K+N), ND–nutrient distribution (homog., heterog.), I–irrigation (normal, increased). Bold P -values indicate significant effects.

Nutrient / Irrigation	<i>R. obtusifolius</i>		<i>A. elatius</i>		<i>T. officinale</i>		<i>T. repens</i>	
	homog.	heterog.	homog.	heterog.	homog.	heterog.	homog.	heterog.
Normal irrigation								
K fertil.	230.6 \pm 10.8	162.7 \pm 69.1	421.6 \pm 77.3	287.3 \pm 56.6	59.6 \pm 8.11	41.0 \pm 5.5	237.2 \pm 35.1	324.8 \pm 95.0
N fertil.	159.8 \pm 30.4	44.0 \pm 11.4	108.7 \pm 30.7	80.9 \pm 12.5	46.3 \pm 3.4	33.3 \pm 1.5	285.9 \pm 32.8	700.1 \pm 51.2
K+N fertil.	98.1 \pm 35.9	134.4 \pm 54.7	142.9 \pm 22.2	68.2 \pm 4.5	56.4 \pm 9.5	79.4 \pm 5.7	296.3 \pm 36.1	197.3 \pm 86.0
Increased irrigation								
K fertil.	132.3 \pm 83.0	74.2 \pm 21.1	315.1 \pm 107.	94.0 \pm 7.2	40.7 \pm 8.5	33.2 \pm 5.1	336.5 \pm 29.6	161.0 \pm 12.1
N fertil.	241.7 \pm 72.8	156.8 \pm 36.7	247.4 \pm 48.1	90.6 \pm 18.4	25.5 \pm 4.7	20.5 \pm 2.4	163.6 \pm 11.2	199.2 \pm 25.3
K+N fertil.	136.6 \pm 81.1	192.0 \pm 57.2	384.1 \pm 87.3	87.7 \pm 8.1	26.8 \pm 4.2	29.5 \pm 7.8	147.7 \pm 33.1	247.6 \pm 42.9
ANOVA results	N	$P = 0.476$	N	$P = \mathbf{0.001}$	N	$P = \mathbf{0.013}$	N	$P = 0.556$
	ND	$P = 0.116$	ND	$P = 0.152$	ND	$P = 0.161$	ND	$P = 0.298$
	I	$P = 0.786$	I	$P < \mathbf{0.001}$	I	$P < \mathbf{0.001}$	I	$P = 0.205$
	N \times ND	$P = 0.630$	N \times ND	$P = 0.181$	N \times ND	$P = 0.903$	N \times ND	$P = 0.675$
	N \times I	$P = 0.302$	N \times I	$P = 0.211$	N \times I	$P = 0.193$	N \times I	$P = 0.215$
	ND \times I	$P = 0.541$	ND \times I	$P = 0.274$	ND \times I	$P = 0.288$	ND \times I	$P = 0.766$

the experiment five soil samples each with 5 g were taken from the soil sections. These soil samples were mixed, air dried, sieved (< 2 mm) and analysed for soil pH, nitrate N, soluble P and soluble K concentrations. Soil pH was measured in CaCl_2 suspension (1:10, mass vol^{-1}) using a glass-electrode. Soil nitrate-N concentration was analysed on 1% K_2SO_4 extracts using a continuous-flow method on a photometer (type 6010; Skalar, Breda, The Netherlands; Hoffmann 1991), available P and K concentrations were determined photometrically after double lactic acid extractions (Riehm 1948).

2.5. Statistical analyses

General patterns between species were first analysed by conducting four-way ANOVAs (factors: species – Spp, 4 levels; nutrient type – NT, 3 levels; nutrient distribution – ND, 2 levels; irrigation – I, 2 levels) using the general linear model procedure in SAS (Version 8.02, SAS Institute, Cary, North Carolina, USA). Then, to test for responses of individual species, three-way ANOVAs were carried out in a similar manner. Data were checked for deviations from normality and for homogeneity of variances prior to analysis.

Table 5. Statistical results on the effect of nutrient type (NT), nutrient distribution (ND) and irrigation (I) on soil potassium and nitrate contents in sections where a particular plant species grew. Bold *P*-values indicate significant effects derived from ANOVAs.

Parameter	NT	ND	I	ND x I	NT x ND	NT x I
Soil K						
<i>R. obtusifolius</i>	< 0.001	< 0.001	0.004	0.215	< 0.001	0.142
<i>A. elatius</i>	< 0.001	< 0.001	< 0.001	0.483	0.031	0.782
<i>T. officinale</i>	< 0.001	< 0.001	< 0.001	0.102	0.034	0.125
<i>T. repens</i>	0.022	< 0.001	< 0.001	0.030	0.441	0.436
Soil NO ₃ -N						
<i>R. obtusifolius</i>	< 0.001	0.001	0.009	0.112	< 0.001	0.317
<i>A. elatius</i>	< 0.001	0.011	0.002	0.102	0.101	0.026
<i>T. officinale</i>	< 0.001	0.120	< 0.001	0.674	0.542	0.010
<i>T. repens</i>	< 0.001	0.088	0.361	0.024	0.701	0.006

3. RESULTS

3.1. General response patterns across species

Overall, tested species differed significantly in their response to irrigation and to a lesser degree to nutrient type and distribution as indicated by various significant interaction terms between species and imposed treatments (Table 2). Irrigation treatments highly significantly altered root morphology, biomass allocation and soil nutrient exploitation across species. The nutrient type used to create patches significantly affected root length, number of root tips, biomass allocation and soil nutrient exploitation while the distribution of nutrients only affected root length, root mass and soil nutrient exploitation of species.

Both shoot and root mass production differed significantly between species. However across species, only root mass was highly significantly affected by all imposed treatments while shoot mass was only affected by nutrient type and irrigation (Table 2). This also

translated into significantly different root:shoot ratios across species that was highly affected by nutrient type and irrigation, but not affected by nutrient distribution.

Generally, soil potassium and nitrate exploitation was similar across plant species but was highly affected by nutrient type, nutrient distribution and irrigation (Table 2). Nutrient exploitation at different nutrient distribution was dependent on irrigation level (e.g., significant ND × I interaction term). Soil pH in soil section was similar between tested species and only affected by irrigation.

3.2. Species-specific responses on root morphology

Root lengths of *R. obtusifolius*, *A. elatius* and *T. officinale* were generally higher when either K or N was used as fertilizers than when both nutrients were combined; *T. repens* root length remained unaffected (Fig. 1). Generally, all species showed significantly greater root lengths at increased irrigation than at regular irrigation. Only root length of *A. elatius* was significantly greater

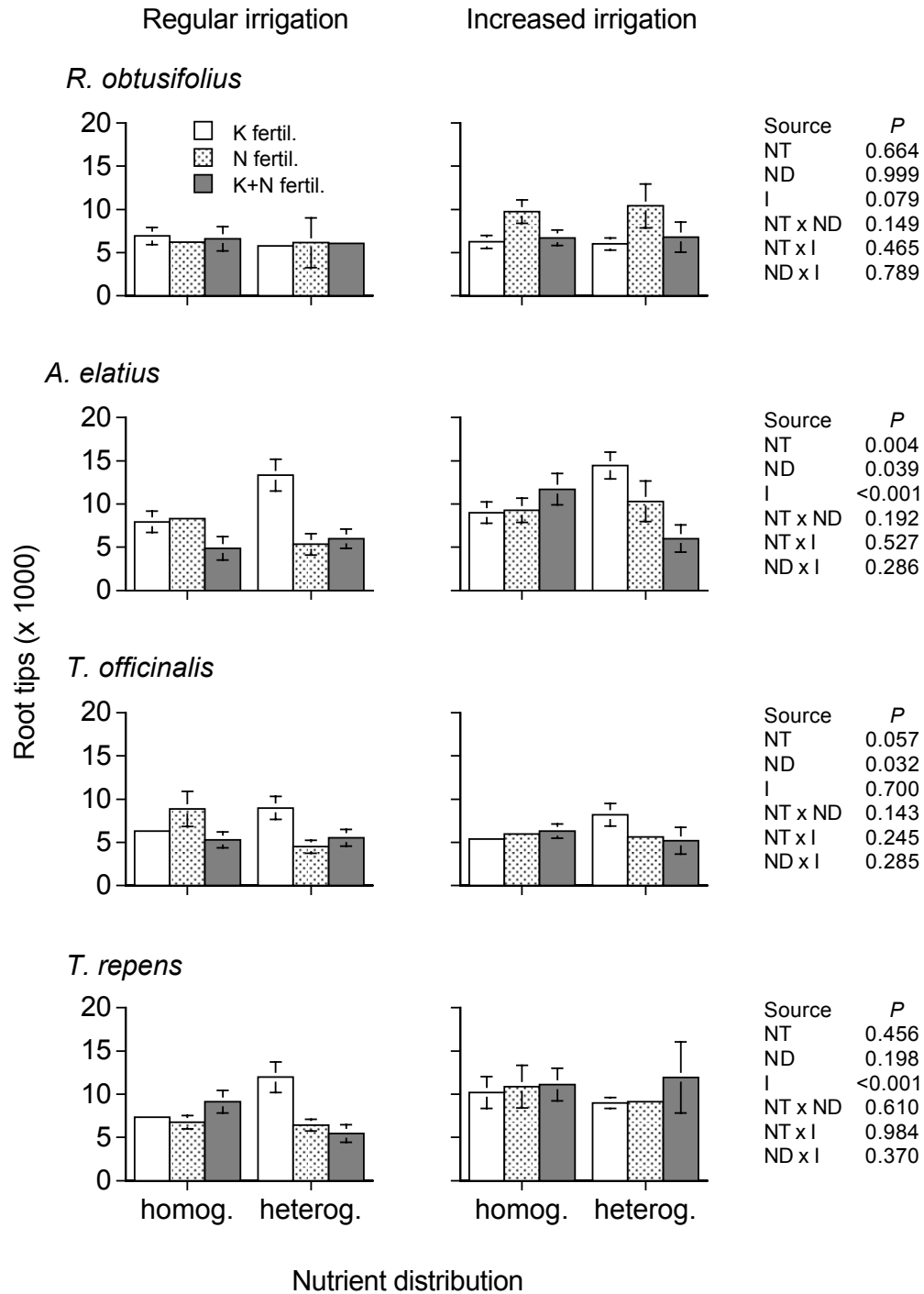


Fig. 2. Number of root tips of grassland species grown in pots with either homogeneously or heterogeneously distributed nutrients (K, N, K+N) under regular or increased irrigation. Means \pm SE (n = 7). P values from ANOVA results with nutrient type (NT), nutrient distribution (ND) and irrigation (I) as factors. Small error bars are not depicted.

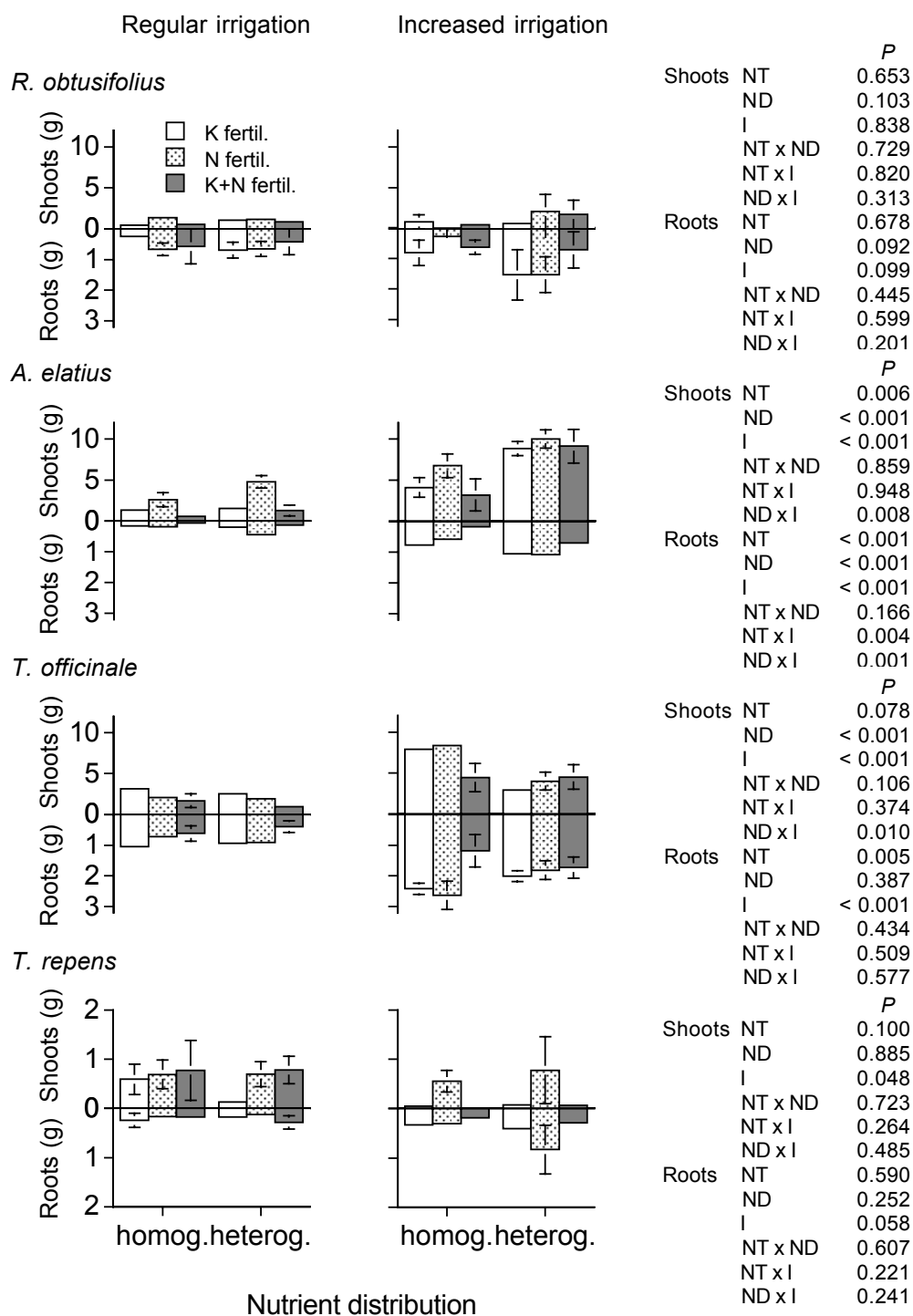


Fig. 3. Shoot and root dry weight of grassland species grown in pots with either homogeneously or heterogeneously distributed nutrients (K, N, K+N) under regular or increased irrigation. Means \pm SE (n = 7). P values from ANOVA results with nutrient type (NT), nutrient distribution (ND) and irrigation (I) as factors. Small error bars are not depicted.

at heterogeneous nutrient distribution than at homogenous distribution while the other species remained unaffected.

Root diameter of *A. elatius* and *T. officinalis* was significantly higher when either K or N was used as fertilizer than when both nutrient types were combined while root diameter of the remaining two species was unaffected (Table 3). Only root diameter of *T. repens* was significantly higher when nutrients were homogeneously distributed than heterogeneously distributed; root diameters of the other species were unaffected by nutrient distribution. *A. elatius* and *T. repens* growing at increased irrigation had significantly thinner roots than at regular irrigation while root diameters of the other species were unaffected by irrigation. Root diameter of *A. elatius* was not only dependent on the nutrient type and distribution but also showed different responses at different irrigation levels (e.g., significant NT \times I and ND \times I interaction terms).

SRL of *A. elatius* was significantly higher when K was used as a fertilizer than when N or K+N were used (Table 4). In contrast, SRL of *T. officinale* was significantly higher when K+N was combined, at heterogeneous than homogenous nutrient distribution, and under increased than regular irrigation. Specific root lengths of *R. obtusifolius* and *T. repens* remained unaffected by type of nutrient used as a fertilizer. Nutrient distribution had no effect on SRL of any species, however increased irrigation led to significantly higher SRL of *A. elatius* but to significantly lower SRL for *T. officinale*; *R. obtusifolius* and *T. officinale* remained unaffected by irrigation.

Number of root tips of *A. elatius* was significantly higher when nutrients were used separately than combined; root tips of the remaining species were similar between different nutrient types (Fig. 2). Both *A. elatius* and *T. officinalis* showed significantly more root tips when nutrients were heterogeneously than homogeneously distributed while the number of root tips of *R. obtusifolius* and *T. repens* were unaffected by nutrient distribution. Increased irrigation led to a significantly higher number of root tips of *A. elatius* and *T. repens* but left number of root tips of *R. obtusifolius* and *T. officinale* unaffected.

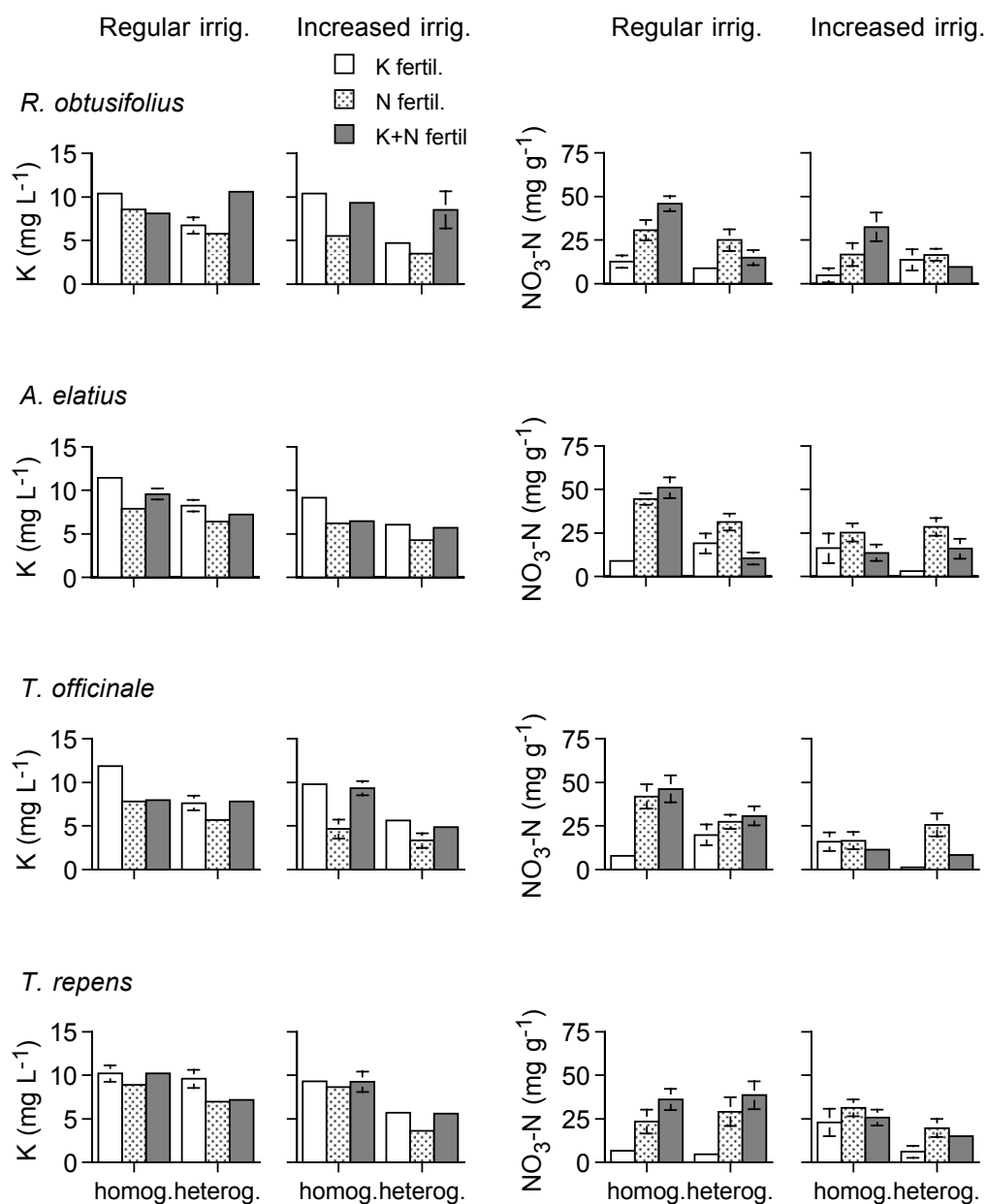
3.3. Species-specific responses on biomass allocation

Neither shoot nor root mass production of *R. obtusifolius* was affected by imposed treatments (Fig. 3). However, root:shoot ratio of *R. obtusifolius* was significantly higher when either K or N was fertilized than when both nutrient types were combined (ANOVA results NT: $P = 0.011$) but remained unaffected by nutrient distribution or irrigation (however significant ND \times I interaction: $P = 0.037$ was found, data not shown).

Shoot mass of *A. elatius* was significantly higher at increased irrigation than at regular irrigation and also when nutrients were distributed heterogeneously than homogeneously and when N was used as a fertilizer compared to the use of K or K+N (Fig. 3). Root mass of *A. elatius* was significantly higher when either K or N was used than when K+N was combined, also heterogeneous distribution led to a higher root mass than homogeneous distribution. Increased irrigation showed higher root mass of *A. elatius* than regular irrigation. Root:shoot ratio of *A. elatius* remained unaffected by imposed treatments (data not shown).

Shoot mass of *T. officinale* was not affected by nutrient type but significantly lower under heterogeneously than homogeneously distributed nutrients (Fig. 3). Increased irrigation led to a significantly higher shoot mass production than at regular irrigation. Root mass production was significantly higher under either K- or N- than K+N-fertilisation. Root:shoot ratio of *T. officinale* was significantly higher when nutrients were homogeneously than heterogeneously distributed ($P < 0.001$) but not affected by nutrient type (however significant ND \times I interaction: $P = 0.031$ was found).

Trifolium repens shoot mass was significantly higher under regular irrigation than increased irrigation; biomass production of *T. repens* remained unaffected by nutrient type and nutrient distribution (Fig. 3). Root:shoot ratio of *T. repens* was significantly lower when N was used as fertilizer compared to the use of K or N+K ($P = 0.011$) and was higher at increased compared to regular irrigation ($P = 0.017$) but not affected by nutrient



Nutrient distribution

Fig. 4. Soil nitrate-nitrogen and potassium concentration under grassland species grown in pots with either homogeneously or heterogeneously distributed nutrients (K, N, K+N) under regular or increased irrigation. Means \pm SE ($n = 7$). See Table 5 for results of statistical analyses. Small error bars are not depicted.

distribution (however significant interaction between ND \times I: $P = 0.021$ was found).

3.4. Species-specific responses on soil nutrient exploitation

For all species, soil potassium exploitation was significantly higher under increased than regular irrigation and significantly higher when nutrients were heterogeneously than homogeneously distributed (Fig. 4, Table 5). However, potassium exploitation by *R. obtusifolius*, *A. elatius* and *T. officinale* was also dependent on nutrient type used and on the distribution of these nutrients (e.g. significant NT \times ND interaction). For *T. repens*, soil potassium exploitation was also significantly affected by nutrient distribution however this was dependent on the irrigation level (e.g. significant ND \times I interaction).

Soil nitrate exploitation of *R. obtusifolius* was significantly lower when both K+N was fertilized than when separate K and N were used; however, this was only the case at homogeneous nutrient distribution (e.g. significant NT \times ND interaction; Fig. 4, Table 5). Soil nitrate exploitation of *A. elatius* and *T. officinale* was significantly affected by nutrient type but was different at different irrigation levels (e.g. significant NT \times I interactions). Nitrate exploitation of *T. repens* was significantly affected by nutrient type and nutrient distribution, however the effect was dependent on the irrigation level (e.g. significant ND \times I and NT \times I interactions).

Soil pH was only affected in sections where either *R. obtusifolius* or *T. officinale* grew; other species did not influence soil pH (data not shown). In sections with *R. obtusifolius*, pH values were significantly higher when nutrients were heterogeneously distributed than homogeneously distributed ($P = 0.005$). Increased irrigation also led to increased soil pH ($P = 0.016$). In sections with *T. officinale*, pH was significantly higher in N- than K- or K+N fertilized soil ($P = 0.010$). Soil receiving increased irrigation showed higher soil pH than regularly irrigated soil ($P < 0.001$; data not shown).

4. DISCUSSION

4.1. Responses of root morphology

Generally, all measures chosen to describe root morphology (root length, root diameter, specific root length, number of root tips) were significantly affected by at least one of the treatments, suggesting that root morphology was a sensitive parameter to determine the influence of nutrient type, soil heterogeneity or irrigation. Root morphology parameters also showed significant interactions between species and treatment factors, indicating that species differed in their response to nutrient types. These species-specific differences in their response might have ramifications for the competitive interactions between *R. obtusifolius* and the grassland species. In the current experiment, species were studied in a competitive environment, however, since the same neighbouring species were used among treatments it is impossible to determine the role of interspecific competition for the observed response patterns.

Focussing on individual effects of species it was surprising to see that in contrast to the hypothesis root morphology of *R. obtusifolius* remained unaffected by different soil nutrient distribution. In contrast, the grass *A. elatius* showed a 40% higher root length production and 6% more root tips, the non-leguminous herb *T. officinale* showed 3% more root tips and the legume *T. repens* a 15% reduced root diameter when nutrients were heterogeneously than homogeneously distributed. Root length production of *R. obtusifolius* was affected by the nutrient type used to fertilize soil and was 30% higher when N and 40% higher when only K was used than when both K+N were combined. Nutrient type also affected root morphology of the grass *A. elatius* by showing a up to twice as high root length and specific root length and twice as many root tips when either N or K was used than when both nutrients were combined. Also, *T. officinale* had a 11% higher root diameter, a 40% higher root length but a 20% lower specific root length when either N or K was used than when both K+N were combined. Root morphology of the legume was not affected by type of nutrients used to create patches. Increased soil

moisture led to a 40% increased root length production of *R. obtusifolius* however did not alter other parameters of root morphology. The other species also showed a high plasticity in root morphology with an averaged 40% increase in root length production, 15% more root tips (despite for *T. officinale* that was unaltered), a 10% higher and 40% lower specific root length for the grass and non-leguminous herb, respectively (*T. repens* remained unaffected).

Based on the number of parameters altered by the treatments, the root system of the grass *A. elatius* showed the highest plasticity, followed by *T. officinale* and *T. repens* with *R. obtusifolius* being the least responsive. This is only partly in correspondence with the notion that coarse-rooted species such as *R. obtusifolius* or *T. officinale* would be less responsive because of higher costs of proliferation to soil microsites than fine-rooted species (such as *A. elatius* or *T. repens*) (Eissenstat 1992, Farley and Fitter 1999). It is tempting to assume that species with a higher root plasticity would have a competitive advantage over species with lower plasticity. However, by comparing the responses of several species to soil nutrient heterogeneity only a weak coupling between root plasticity and plant performance was found (Johnson and Biondini 2001). Additionally little support for the widespread assumption that foraging precision increases the benefit gained from growth in heterogeneous soil could be found and thus the ecological relevance of small-scale nutrient heterogeneity in natural systems was questioned (Kembel and Cahill 2005). These findings suggest that while roots may rapidly respond to the presence of nutrient patches, this may not translate immediately into plant performance. It is important to note, however, that most studies on the response of plants to soil nutrient heterogeneity excluded competition between species. Thus, it is very likely that in a mixed plant community the benefits of root plasticity might be more critical especially for subdominant species like *A. elatius*, *T. officinale* and *T. repens* as a general adaptation to compete for soil nutrients regardless of the extent of soil nutrient heterogeneity (Johnson and Biondini 2001). In the current study, all species tested seemed to have suf-

ficient plasticity in the root system to track the scale of the soil heterogeneity and that this can at least for *A. elatius* and *T. officinale* translate into altered shoot and root biomass production. Results also showed that many interactions between nutrient distribution and irrigation could be observed, indicating that irrigation affects the accessibility of the nutrient in the patches relative to homogeneous distribution and that this can also lead to a higher rate of nutrient exploitation in pots receiving increased irrigation.

For the current study it was assumed that responses to nutrient distribution would occur due to inherent differences in a plants' root system and that plants with adventitious root systems (*A. elatius*, *T. repens*) would differ in their response to the treatments than plants with a taproot system (*R. obtusifolius*, *T. officinale*). Data suggest that the basic architecture of the root system is not necessarily a good indicator of a plants' response to soil heterogeneity or soil moisture. This could also be due to the finding that the response to soil heterogeneity is not a fixed species trait but one that can vary considerably with plant developmental stage (Hutchings and John 2004). Therefore, seedlings could be considered to be more affected by small-scale heterogeneity than mature plants with greater root systems. Another aspect for a plants' response to soil heterogeneity might also be its dependence on mycorrhizal associations (Farley and Fitter 1999). Indeed, *R. obtusifolius* is the only species in the current investigation that combines two traits that could be a reason for its non-responsiveness to nutrient heterogeneity: no mycorrhization and a coarse root system. The other more responsive species tested showed either thinner root system (*A. elatius*) and/or potential association with mycorrhizal fungi (*A. elatius*, *T. officinale*, *T. repens*). The little response of the legume to soil N heterogeneity might also be explained by its independency from soil N due to N-fixation. In fact, no root nodules of nitrogen fixing bacteria could be observed at harvesting indicating that the duration of the experiment was not long enough to allow N-fixing bacteria to develop. Alternatively, although root growth is the most visually obvious response to soil heterogeneity also

physiological shifts in uptake kinetics could be more important in determining growth responses to soil heterogeneity (Jackson *et al.* 1990, Hodge 2004).

4.2. Biomass allocation and nutrient exploitation

Biomass allocation was significantly affected by treatments and varied considerably between species. According to Campbell *et al.* (1991) *R. obtusifolius* would be considered a dominant plant characterized by reallocating biomass towards roots and developing an extensive root system by capturing and monopolizing a large portion of soil resources through a coarse scale foraging strategy. Contrary, the subdominant species are thought to capture soil resources through a fine scale foraging strategy with little changes in their root biomass. However, in the current study biomass allocation of *R. obtusifolius* was unaltered by soil heterogeneity and only affected by nutrient type by allocating significantly more biomass to the root system than to the shoots when either N or N+K was used but remained unaffected by K fertilisation. In contrast the subdominant species *A. elatius* and *T. officinale* showed manifold responses to soil heterogeneity in their above- and belowground biomass production. Especially shoot mass of *A. elatius* and *T. officinale* was significantly lower at heterogeneous than at homogeneous nutrient distribution while *R. obtusifolius* and *T. repens* shoot mass remained unaffected. This might have consequences for the competitive relationships between species in the grassland community, although in an other experiment it could be shown that biomass allocation of *R. obtusifolius* was rather insensitive to shoot competition through grassland species (Zaller 2004a).

Generally the size of the root system may have important implications for the performance of these species in grassland communities since species with a large root system are likely to have a greater ability to exploit nutrient-rich patches than those with small root systems (Farley and Fitter 1999). The current results showed that although *T. officinale* had the largest root biomass across treatments (followed by *R. obtusifolius*, *T.*

repens and *A. elatius*) and *T. repens* had the greatest root length (followed by *A. elatius*, *R. obtusifolius* and *T. officinale*) - these species were among the least responsive. This indicates that other parameters than root morphology or biomass allocation are likely to influence response patterns. Given the species-specific responses of root systems to treatments, it was interesting to see that species did not differ in the exploitation of soil potassium and nitrate-nitrogen. However, because species varied considerably in their individual response to nutrient type, distribution and irrigation as indicated by significant interactions between treatment factors and species it is difficult to draw any general response patterns. In terms of nitrate-nitrogen exploitation only soil sections with *T. officinale*, the species with greatest root biomass, were unaffected by nutrient distribution; soil nitrate-nitrogen contents under *T. repens* was neither affected by nutrient distribution nor by irrigation. This indicates that nutrient type, soil heterogeneity and soil moisture levels can alter the root morphology and allocation of species but have only little influence on soil nutrient exploitation. Explanations for this could be in different capacities to store nutrients in roots (Gebauer *et al.* 1984, Zaller 2004a) or adjustments at the physiological level by influencing nutrient uptake kinetics (Jackson *et al.* 1990, Hodge 2004).

One of the central aims of this study was to compare the response of *R. obtusifolius* with that of other co-occurring grassland species. Results showed that *R. obtusifolius* was rather insensitive to soil heterogeneity, but responded to single use of either N or K. Overall, the grass and the non-leguminous herb showed a high plasticity, while the leguminous species remained rather unresponsive. Soil moisture levels have been shown to significantly influence the response of species to soil heterogeneity making difficult to predict species responses in a natural environment with fluctuating soil moisture. Results from this study suggest that, considering the parameters measured in this study, *R. obtusifolius* does not seem to have superior traits to utilize soil nutrient heterogeneity, certain nutrient types or higher soil moisture that fundamentally differentiates it

from the other grassland species tested. This also implies that management measures oriented on the improvement of the competitive ability of co-occurring grassland species (e.g. sheep grazing, Zaller 2006b) could also be important in soils with heterogeneous nutrient distribution and higher soil moisture. Finally, results also show that the functional ecology of *R. obtusifolius* is still very poorly understood and that further research is especially needed on interactions in multi-species communities in order to develop strategies for the sustainable management of this species (see also Zaller 2004b).

ACKNOWLEDGEMENTS: I am very grateful to Alexandra Donati, Harriet Leese, Britta Staffell, Christina Günther, Sonja Reinhardt, Henning Riebeling, Ute Schlee, Johannes Siebigtheroth, Birgit Stöcker, Benedick Roy Usher and Dieter Zedow for their excellent help in the laboratory and greenhouse.

5. REFERENCES

- Bilbrough C.J., Caldwell M.M. 1995 – The effects of shading and N status on root proliferation in nutrient patches by the perennial grass *Agropyron desertorum* in the field – *Oecologia*, 103: 10–16.
- Caldwell M.M., Manwaring J.H., Durham S.L. 1991 – The microscale distribution of neighbouring plant roots in fertile soil microsites – *Funct. Ecol.*, 5: 765–772.
- Campbell B.D., Grime J.P., Mackey J.M.L. 1991 – A trade-off between scale and precision in resource foraging – *Oecologia*, 87: 532–538.
- Cavers P.B., Harper J.L. 1964 – Biological flora of the British Isles. *Rumex obtusifolius* L. and *Rumex crispus* L. – *J. Ecol.*, 52: 737–766.
- Crick J.C., Grime J.P. 1987 – Morphological plasticity and mineral nutrient capture in two herbaceous species of contrasted ecology – *New Phytol.*, 107: 403–414.
- Day K.J., Hutchings M.J., John E.A. 2003a – The effects of spatial pattern of nutrient supply on the early stages of growth in plant populations – *J. Ecol.*, 91: 305–315.
- Day K.J., John E.A., Hutchings M.J. 2003b – The effects of spatially heterogeneous nutrient supply on yield, intensity of competition and root placement patterns in *Briza media* and *Festuca ovina* – *Funct. Ecol.*, 17: 454–463.
- Dickinson C.H., Craig G. 1990 – Effects of water on the decomposition and release of nutrients from cow pats – *New Phytol.*, 115: 139–147.
- Eissenstat D. 1992 – Costs and benefits of construction roots of small diameter – *Journal of Plant Nutrition*, 15: 763–782.
- Ellenberg H. 1986 – *Vegetation Mitteleuropas mit den Alpen*. 4th edn, Verlag Eugen Ulmer, Stuttgart. (in German)
- Facelli E., Facelli J.M. 2002 – Soil phosphorus heterogeneity and mycorrhizal symbiosis regulate plant intra-specific competition and size distribution – *Oecologia*, 133: 54–61.
- Farley R.A., Fitter A.H. 1999 – The response of seven co-occurring woodland herbaceous perennials to localized nutrient-rich patches – *J. Ecol.*, 87: 849–859.
- Fransen B., De Kroon H., Berendse F. 1998 – Root morphological plasticity and nutrient acquisition of perennial grass species from habitats of different nutrient availability – *Oecologia*, 115: 351–358.
- Fransen B., De Kroon H., Berendse F. 2001 – Soil nutrient heterogeneity alters competition between two perennial grass species – *Ecology*, 82: 2534–2546.
- Gebauer G., Melzer A., Rehder H. 1984 – Nitrate content and nitrate reductase activity in *Rumex obtusifolius* L. I. Differences in organs and diurnal changes – *Oecologia*, 63: 136–142.
- Gross K.L., Peters A., Pregitzer K.S. 1993 – Fine root growth and demographic responses to nutrient patches in four old-field plant species – *Oecologia*, 95: 61–64.
- Gross K.L., Pregitzer K.S., Burton A.J. 1995 – Spatial variation in nitrogen availability in three successional plant communities – *J. Ecol.*, 83: 357–367.
- Hodge A. 2004 – The plastic plant: root responses to heterogeneous supplies of nutrients – *New Phytol.*, 162: 9–24.
- Hoffmann G. 1991 – *Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten Methodenbuch. Band I. Die Untersuchung von Böden*. 4th edn – VDLUFA-Verlag, Darmstadt, Germany. (in German)
- Hutchings M.J., John E.A. 2004 – The effects of environmental heterogeneity on root growth and root/shoot partitioning – *Ann. Bot.*, 94: 1–8.
- Jackson R.B., Caldwell M.M. 1989 – The timing and degree of root proliferation in fertile-soil microsites for three cold-desert perennials – *Oecologia*, 81: 149–153.
- Jackson R.B., Caldwell M.M. 1991 – Kinetic responses of *Pseudoroegneria* roots to

- localized soil enrichment – *Plant Soil*, 138: 231–238.
- Jackson R.B., Caldwell M.M. 1993 – The scale of nutrient heterogeneity around individual plants and its quantification with geostatistics – *Ecology*, 74: 612–614.
- Jackson R.B., Manwaring J.H., Caldwell M.M. 1990 – Rapid physiological adjustment of roots to localized soil enrichment – *Nature*, 344: 58–60.
- Johnson H.A., Biondini M.E. 2001 – Root morphological plasticity and nitrogen uptake of 59 plant species from the Great Plains grasslands, U.S.A. – *Basic Appl. Ecol.*, 2: 127–143.
- Kembel S.W., Cahill J.F. 2005 – Plant phenotypic plasticity belowground: A phylogenetic perspective on root foraging trade-offs – *Am. Natur.*, 166: 216–230.
- Kutschera L., Sobotik M. 1985 – Gülleflora – Unterschiede durch Klima und Boden. (In: 7. Arbeitstag, BAL Gumpenstein, Ed. – BAL Gumpenstein – Gumpenstein, Austria, pp. 79–119. (in German))
- Pino J., Haggard R.J., Sans F.X., Masalles R.M., Hamilton R.N.S., Sackville Hamilton R.N. 1995 – Clonal growth and fragment regeneration of *Rumex obtusifolius* L – *Weed Res.*, 35: 141–148.
- Pregitzer K.S., Hendrick R.L., Fogel R. 1993 – The demography of fine roots in response to patches of water and nitrogen – *New Phytol.*, 125: 575–580.
- Rehder H. 1982 – Nitrogen relations of ruderal communities (Rumicion alpini) in the Northern Calcareous Alps – *Oecologia*, 55: 120–129.
- Reynolds H.L., D'Antonio C. 1996 – The ecological significance of plasticity in root weight ratio in response to nitrogen: opinion – *Plant Soil*, 185: 75–97.
- Riehm H. 1948 – Arbeitsvorschrift zur Bestimmung der Phosphorsäure und des Kaliums nach Lactatverfahren – *Z. Pflanzenähr. Düng. Bodenk.*, 40: 152–156. (in German)
- Robinson D., Rorison I. H. 1983 – A comparison of the responses of *Lolium perenne* L., *Holcus lanatus* L. and *Deschampsia flexuosa* (L.) Trin. to a localized supply of nitrogen – *New Phytol.*, 94: 263–273.
- Robinson D., van Vuuren M.M.I. 1998 – Responses of wild plants to nutrient patches in relation to growth rate and life-form (In: Inherent variation in plant growth. Physiological mechanisms and ecological consequences, Eds: H. Lambers, H. Poorter M.M.I. Van Vuuren) – Backhuys Publishers, Leiden, The Netherlands, pp. 237–257.
- Ryel R.J., Caldwell M.M., Manwaring J.H. 1996 – Temporal dynamics of soil spatial heterogeneity in sagebrush-wheatgrass steppe during a growing season – *Plant Soil*, 184: 299–309.
- Van Vuuren M.M.I., Robinson D., Griffiths B.S. 1996 – Nutrient inflow and root proliferation during the exploitation of a temporally and spatially discrete source of nitrogen in soil – *Plant Soil*, 178: 185–192.
- Wijesinghe D.K., John E.A., Beurskens S., Hutchings M.J. 2001 – Root system size and precision in nutrient foraging: responses to spatial pattern of nutrient supply in six herbaceous species – *J. Ecol.*, 89: 972–983.
- Wijesinghe D.K., John E.A., Hutchings M.J. 2005 – Does pattern of soil resource heterogeneity determine plant community structure? An experimental investigation – *J. Ecol.*, 93: 99–112.
- Zaller J.G. 2004a – Competitive ability of *Rumex obtusifolius* against native grassland species: above- and belowground allocation of biomass and nutrients – *J. Plant Diseases Protect.*, Spec. Iss. XIX: 345–351.
- Zaller J.G. 2004b – Ecology and non-chemical control of *Rumex crispus* and *R. obtusifolius* (Polygonaceae): a review – *Weed Res.*, 44: 414–432.
- Zaller J.G. 2006a – Allelopathic effects of *Rumex obtusifolius* leaf extracts against native grassland species – *J. Plant Diseases Protect.*, Spec. Iss. XX: 463–470.
- Zaller J.G. 2006b – Sheep grazing vs. cutting: regeneration and soil nutrient exploitation of the grassland weed *Rumex obtusifolius* – *Bio-Control*, 51: 837–850.
- Zaller J.G., Köpke U. 2004 – Effects of traditional and biodynamic farmyard manure amendment on yields, soil chemical, biochemical and biological properties in a long-term field experiment – *Biol. Fertil. Soils*, 40: 222–229.

Received after revising May 2007