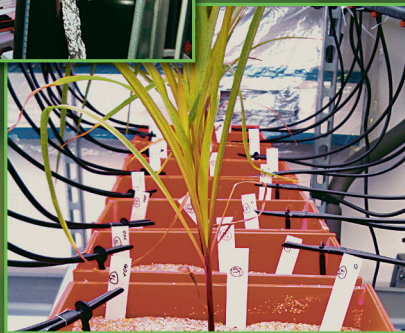


The impact of nutrient heterogeneity on maize plants

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Abbreviations.....	VIII
Zusammenfassung.....	X
Abstract	XII
1. Introduction.....	1
1.1 Heterogeneity	1
1.2 Current state of research.....	2
1.3 Hypotheses and Questions	5
2. Materials and Methods.....	6
2.1 Plant material	6
2.2 Growth Conditions.....	6
2.3 Split root pots	6
2.4 Irrigation system	7
2.5 Nutrient solution	8
2.5.1 Steady-state nutrition and relative growth rate (RGR).....	8
2.5.2 Experiment for determination of the RGR of maize.....	9
2.6 Harvest of the plants	13
2.7 Structural and functional Parameters	13
2.7.1 Structural Parameters.....	14
2.7.1.1 Development of the plants.....	14
2.7.1.2 Biomass.....	14
2.7.1.3 Root structure	15
2.7.1.4 Leaf area	15
2.7.2 Functional Parameters	15
2.7.2.1 Photosynthesis	15
2.7.2.2 Metabolites	16
2.7.2.2.1 Extraction of chlorophyll and carbohydrate.....	16
2.7.2.2.2 Chlorophyll.....	17
2.7.2.2.3 Soluble carbohydrates.....	17
2.7.2.2.4 Starch	19
2.7.2.2.5 Free amino acids	19
2.7.2.3 C, N and S content	20
2.8 Statistics	21
3. Results	22
3.1 Structural parameters.....	22
3.1.1 Development of the plants in the experiments: structural measurements.....	22
3.1.1.1 Number and average actual state of the leaves	22

3.1.1.2 Leaf area	24
3.1.3 Biomass.....	24
3.1.3.2 Total dry matter	24
3.1.3.3 Shoot biomass.....	25
3.1.3.4 Root biomass.....	26
3.1.3.5 Shoot/root ratio	28
3.1.4 Root structure.....	29
3.1.4.1 Root surfaces	29
3.1.4.2 Root length	30
3.1.4.3 Specific root length	31
3.1.5 Findings of the structural parameters	33
3.2 Functional Parameters	35
3.2.1 Development of the plants in the experiments: functional measurements	35
3.2.1.1 Photosynthesis	35
3.2.1.2 Chlorophyll.....	36
3.2.2 Internal nutrient concentration.....	36
3.2.2.1 C/N ratio	36
3.2.2.2 C/S ratio.....	44
3.2.3 Metabolites	47
3.2.3.1 Sugars and Starch.....	47
3.2.3.2 Free amino acids	52
3.2.4 Findings of the functional parameters	53
3.3 Summary of structural and functional parameters.....	54
4. Discussion	56
4.1 Development of the plants during the experiments	56
4.2 Impact of heterogeneously supplied nutrients on biomass	57
4.3 Impact of nutrient heterogeneity on the internal nutrient concentration of the plants....	59
4.3.1 Carbon.....	59
4.3.2 Nitrogen	60
4.3.3 Sulphur	61
4.4 Regulation of root proliferation	62
4.5 Effect of nutrient heterogeneity on photosynthesis and chlorophyll concentration.....	62
4.5.1 Photosynthesis	62
4.5.2 Chlorophyll.....	63
4.6 Impact of heterogeneously distributed nutrients on metabolites in the plants	63
4.6.1 Sugar and starch	63
4.6.2 Free amino acids.....	64

4.7 Conclusion.....	65
4.8 Outlook	65
5. References	67

Abbreviations

AASL	average actual state of leaves
ALA	alanine
ANOVA	Analysis of variance
ASL	actual state of the different leaves of the plants in the respective treatment
ASN	asparagine
ASP	aspartate
ATP	Adenosin-triphosphate
C _{chl}	chlorophyll concentration [mg*g ⁻¹ FW]
C _{hexose}	carbohydrate concentration [μmol g ⁻¹]
C _{starch (glucose)}	concentration of starch (glucose) [μmol*g ⁻¹]
F _{m Plant}	plant fresh matter [g]
GLN	glutamine
GLU	glutamate
GLY	glycine
HATS	high affinity transport systems
HIS	histidine
ICP-OES	Inductively Coupled Plasma with Optical Emission Spectroscopy
ILEU	isoleucine
k	ratio of plant fresh/dry matter
K _m	the affinity of the transporters to the nutrient ion
L	number of leaves of the plants in the respective treatment
LATS	low affinity transport systems
LEU	leucine
LYS	lysine
MET	methione
m _{FW}	fresh weight of the plant material [mg]
N(%) _{Dm}	proportional amount of nitrogen in plant dry matter
N(%) _{Fm Plant}	proportional amount of nitrogen in plant fresh matter
NADP ⁺	Nicotine-amid-dinucleotidphosphate
N _{Fm Plant}	nitrogen content in plant fresh matter [g]
OD	optical density
p	level of significance
p.	page
PHE	phenylalanine
PRO	proline
PVC	polyvinylchloride

r.h.	relative humidity
RAR	relative addition rate
RGR	relative growth rate
RL	root length [m]
RUR	relative uptake rate
SER	serine
SRL	specific root length [m/g]
THR	threonine
TNC	total non-structural carbohydrate
TRDM	total root dry matter [g]
TRP	tryptophan
TYR	tyrosine
VAL	valin
V_{aliquot}	volume of the aliquot [μl]
V_{cuvette}	volume of the cuvette [μl]
V_{extract}	volume of the extract [μl]
V_{inkub}	incubation volume [μl]
$V_{\text{inkub-aliquot}}$	incubated extract volume [μl]
W	biomass of the plant [g]
ZCH	Central Division of Analytical Chemistry

Zusammenfassung

In dieser Arbeit wurden sowohl strukturelle als auch funktionelle Reaktionen der Pflanze auf heterogen verteilte Nährstoffe untersucht. In Splitroot-Experimenten mit *Zea mays* hybrid *Helix* erhielten alle Pflanzen dieselbe Menge an Nährstoffen, welche aber unterschiedlich verteilt waren (0.5/0.5, 0.6/0.4, 0.7/0.3, 0.8/0.2, 0.9/0.1, 1.0/0.0). Die Menge der zugegebenen Nährstoffe war an die relative Wuchsrate (RGR) angepasst, um eine konstante, dem Pflanzenbedarf angepasste Ernährung zu gewährleisten. Zum einen wurde die Menge der Nährstoffe an das 1,5-fache der RGR angepasst und zum anderen auf das 0,5-fache der relativen Wuchsrate reduziert. So war es erstmals möglich zu untersuchen, ab welcher ungleichen Nährstoffverteilung die Pflanzen reagierten und ob diese Reaktion vom Ernährungsstatus der Pflanzen abhängig war. Des Weiteren wurde untersucht, ob die Pflanzen lokal oder auf der Ebene der Gesamtpflanze auf Nährstoffheterogenität reagierten. Auf der Ebene der Biomassen konnte gezeigt werden, dass Pflanzen lokal auf Nährstoffheterogenität reagierten. Diese Reaktion war abhängig vom Ernährungsstatus der Pflanze, denn gut ernährte Pflanzen bildeten schon bei einem Nährstoffverhältnis von 0.6/0.4 unterschiedliche Wurzelbiomassen. Pflanzen unter Nährstoffmangel taten dies erst bei einer Ungleichverteilung von 0.7/0.3. Für diese unterschiedliche Reaktion schien jedoch nicht das Nährstoffverhältnis, sondern möglicherweise der Konzentrationsunterschied an zugegebenem Nitrat verantwortlich zu sein. Wenn der Konzentrationsunterschied über mehrere Tage (> 4 Tage) andauerte, reagierte die Pflanze. Nährstoffheterogenität hatte keinen Einfluss auf die Gesamt-, Gesamtwurzel- bzw. Sprossbiomasse oder das Spross/Wurzel Verhältnis. Allerdings wurden die Biomassen des Sprosses vom Ernährungsstatus der Pflanzen beeinflusst. Gut ernährte Pflanzen hatten höhere Sprossbiomassen als Pflanzen unter Nährstoffmangel. Dies wurde auch in den unterschiedlichen Spross/Wurzel Verhältnissen beider Versuche deutlich.

In dieser Arbeit wurden verschiedene funktionelle Parameter unter heterogenen Nährstoffbedingungen untersucht. Weder die Photosyntheserate noch die Zucker- und Stärkekonzentrationen in den Wurzeln und in den Blättern waren beeinflusst. Allerdings stieg mit ansteigender Heterogenität die Konzentration an Stickstoff im oberirdischen Pflanzenteil wie auch in der Gesamtpflanze an. Im Gegensatz dazu waren die Konzentrationen an Schwefel in der Pflanze durch das heterogene Nährstoffangebot unbeeinflusst. Sowohl der durch ansteigende Heterogenität verursachte Anstieg der Stickstoffkonzentration als auch die gleich bleibenden Schwefelkonzentrationen ließen sich bei gut ernährten Pflanzen und bei Mangelpflanzen nachweisen. Diese Reaktionen waren durch die unterschiedlichen Aufnahmesysteme für Nitrat und Sulfat und deren räumliche Verteilung in der Wurzel zu erklären. Eine weitere lokale, funktionelle Reaktion auf Nährstoffheterogenität stellte die Beeinflussung der Aminosäurekonzentrationen in den Blättern dar. Unabhängig vom

Nährstoffstatus wiesen die Kontrollvarianten (0.5/0.5) gegenüber der Variante 1.0/0.0 signifikant geringere Gesamtamino­säurekonzentrationen auf.

Es konnte in dieser Arbeit nachgewiesen werden, dass die Pflanzen oft auf die Gesamtmenge der angebotenen Nährstoffe stärker reagierten als auf deren Verteilung. Wenn die Pflanzen allerdings auf heterogene Nährstoffverteilung reagierten, so wurden strukturelle als auch funktionelle Reaktionen vom Nährstoffstatus der Pflanzen beeinflusst. Die Reaktionen der Pflanzen auf Nährstoffheterogenität waren auf der lokalen und auf der Ebene der Gesamtpflanze zu finden.

Abstract

In this work the impact of heterogeneously distributed nutrients on plants was examined. In splitroot experiments *Zea mays* plants (*Zea mays* hybrid *Helix*) received the same amounts of nutrients, with different fractions supplied to each root compartment (0.5/0.5, 0.6/0.4, 0.7/0.3, 0.8/0.2, 0.9/0.1, 1.0/0.0). The amounts of nutrients were adapted to the relative growth rate (RGR) of this species in order to achieve a nutritional supply adapted to demand. Two different experiments were run. In one experiment the amount of nutrients was adapted to 1.5*RGR, in another experiment to 0.5*RGR. With this experimental design, it was possible to measure at what level of nutrient heterogeneity plants responded and if these responses were influenced by the total amounts of given nutrients. In addition, the impact of nutrient heterogeneity on structural and functional parameters was measured.

The experiments indicated that plants responded locally to nutrient heterogeneity by changes in biomass, and that this response depended on the nutrient status of the plants. Well fertilized plants responded with different root biomass at a ratio of nutrient supply of 0.6/0.4. In contrast to this, plants with a lack of nutrients responded to an unequal supply of nutrients in the 0.7/0.3 treatment. The ratio of given nutrients in the root compartments were probably not responsible for this different reaction, however, because the response was possibly caused by a minimum difference of supplied nitrate concentrations in the split root sides within the treatments. If this difference in concentration was exceeded long enough (> 4 days), plants responded. Nutrient heterogeneity had no impact on the total plant, total root and shoot biomass. But the nutrition status of the plants influenced the shoot biomass. Well fertilized plants had higher shoot biomass than plants under nutrient deprivation. This was detectable in different shoot/root ratios, too.

In this work different functional parameters of plants with heterogeneous nutrient supply were measured. Neither the rate of photosynthesis nor the sugar and starch concentrations in leaves and roots were influenced. On the other hand with increasing heterogeneity, the concentration of nitrogen in the above ground part of the plants as well as in the total plant increased. In contrast to this, the sulphur concentrations in the plants were not influenced. This could be explained with different uptake systems for nitrogen and sulphate and their distribution in the roots. Furthermore the amino acid concentration in the leaves was influenced by the heterogeneous distribution of supplied nutrients. Irrespective of the nutritional status of the plants, the control treatments (0.5/0.5) showed lower total amino acid concentrations compared to the 1.0/0.0 treatment.

In this work it was demonstrated that the amount of supplied nutrients had a stronger impact on plant behaviour than their distribution. If plants responded structurally as well as functionally to nutrient heterogeneity, these responses were partly influenced by the nutritional status of the plants, occurred locally and at the whole plant level.

1. Introduction

1.1 Heterogeneity

Plants and animals are living in a manifold and dynamic environment which is formed by locally varying factors. This environment is formed by natural processes (e.g. geological processes) as well as anthropogenic processes (e.g. urban development or pollution) which results in a spatially heterogeneous environment. Plants have developed in this heterogeneous environment before anthropogenic processes started and are to this day able to survive, to grow and to propagate. How plants as sessile organisms can manage to prepare for and respond to this heterogeneous environment has been of scientific interest for a long time, and is still poorly understood.

Because the term heterogeneity is often used in biology, it has to be defined at the beginning of this work. The term seems to be simple when contrasted with homogeneity. Homogeneity can be defined as the absence of variation.

It is often stated that heterogeneity can happen in time and space (Stark, 1994; Hutchings & Wijesinghe, 1997; Hodge, 2004). *Temporal heterogeneity* is given, if a chosen factor differs in time but is constant in space. For example, temporal heterogeneity is given, if in a considered period (e.g. one year) the vegetation cover of an area is changed. The term *temporal heterogeneity* is sometimes synonymously used with the term *variability* (Shachak & Brand, 1991).

Spatial heterogeneity exists if a chosen factor assumes different values at different locations (Kolasa & Rollo, 1991). For example, spatial heterogeneity is given, if the age class distribution of one tree species in different regions is compared. In other publications *spatial heterogeneity* is synonymously used with the term *heterogeneity* (Shachak & Brand, 1991). Sometimes temporal and spatial heterogeneity can not be separated, because if two locations differ in their temporal variation (temporal heterogeneity), they represent spatial heterogeneity.

It is possible to define heterogeneity in another way. One can distinguish between *measured* and *functional heterogeneity* (Kolasa & Rollo, 1991). *Measured heterogeneity* is defined as arbitrary measurements (e.g. distance in cm, km or pH in soil) which describe differences. Such differences are not necessarily relevant for the organisms of interest (e.g. individuals, populations, etc.). For example, several measurements of pH in soil are done within 1m² and show varying data. It does not follow that this heterogeneous distribution of pH is relevant for example for the fitness of large trees. In contrast to this, *functional heterogeneity* is defined as heterogeneity which organisms (e.g. in individuals, populations, etc.) perceive and respond to. *Functional heterogeneity* can be spatial and temporal.

This work deals with the impact of spatial heterogeneity of nutrient availability on plant performance. Therefore, in this work nutrient heterogeneity is defined as functional and as a spatial heterogeneity which is constant over time.

1.2 Current state of research

The impact of nutrient heterogeneity on plants has been investigated in field and laboratory experiments for crops as well as for non-food plants. The response of young maize plants (*Zea mays*) to nutrient heterogeneity is first described in the middle of the 19th century: plants have developed more roots in nutrient rich patches than in nutrient poor soil (Nobbe, 1862).

In an elementary experiment the effect of local nutrient enrichment on root growth is tested by Drew *et al.* (1973). Single root axes of barley (*Hordeum vulgare* L.) were grown into three compartments filled with sand. In these compartments the concentration of nutrients were controlled separately. High nitrate concentrations were given in one compartment and promoted the formation of more first and second order laterals per unit primary root length within this compartment. Furthermore, this high nitrate compartment has shown a greater lateral root formation. Later, the same effect on root growth was shown in response to a high local concentration of phosphate, but was absent, when potassium concentration was increased locally (Drew, 1975a). Drew has shown that barley plants respond in the same morphological way to nitrate as to phosphate. Later these responses were confirmed for plants such as maize and wheat in different experiments (Anghinoni & Barber, 1980; Dejager, 1982; Granato & Raper, 1989; Sattelmacher & Thoms, 1995; vanVuuren *et al.*, 1996) and a theoretical paper (Robinson, 1996).

That plants respond to local enhanced concentrations of nitrate and phosphate in the same way is of interest, because nitrate and phosphate have different mobility in soil. Nitrate is more mobile than phosphate. Phosphate diffuses slowly in soil, because of its exchange reaction with soil particles (Brümmer *et al.*, 2002). Why plants respond in the same morphological way for a mobile ion like nitrate as for an immobile ion like phosphate is still poorly understood.

Maize plants show an increased uptake rate for potassium, phosphate or nitrate if these nutrients are locally supplied in a high concentration (Granato & Raper, 1989; Thoms, 1992; Sattelmacher & Thoms, 1995). In a ¹⁵N labelled organic patch, roots of wheat plants only capture 8 % of the N in the first 5 days of exploitation. Within the following 7 days roots proliferate and capture 63 % of N (vanVuuren *et al.*, 1996).

There are two different types of uptake systems for nitrate: a high affinity and a low affinity transport system (Smith *et al.*, 2000; Orsel *et al.*, 2002). Generally, high affinity transport systems (HATS) are operating if the external nutrient concentration is low. Low affinity

transport systems (LATS) are operating if the external nutrient concentration is high. If diffusion of the nutrients to the root surface is not limiting nutrient uptake, nutrient uptake is similar to the Michaelis-Menten kinetics. The maximal inflow rate (according to $V_{\max}$ in Michaelis-Menten kinetics) can be used as a description of root capacity for ion uptake.

K_m describes the affinity of the transporters to the nutrient ion (according to substrate concentration at $V_{\max}/2$ in Michaelis-Menten kinetics). A low K_m confers a high and a high K_m a low affinity (Lambers *et al.*, 1998).

One high and two low affinity transport systems can be responsible for nitrate uptake (Glass, 2002) depending on nitrate concentration. If the nitrate concentration in the soil solution is high, the low affinity transport system is responsible for nitrate uptake. At low nitrate concentrations only the high affinity transport systems are involved in the nitrate uptake (Quaggiotti *et al.*, 2003).

Nitrate is not only a nutrient but also a signal for plant growth. It regulates nitrate assimilation, but light, cytokinin, CO_2 levels, circadian rhythms, carbon and nitrogen metabolites such as sucrose and glutamine play regulatory roles as well (Crawford, 1995).

After an NO_3^- inducible Arabidopsis gene (ANR1) is identified and this gene indicate to be determinant for developmental plasticity in Arabidopsis roots (Zhang & Forde, 1998), a dual pathway model for regulation of lateral root growth and development by nitrate is presented (Zhang *et al.*, 1999). In this model root branching is influenced by two different signals. One signal is the plant's internal N status and the other signal the external supply of nitrate.

There are two possibilities to regulate the response on a local increased nitrate concentration. The response can be local or systemic. The local regulation of lateral root growth as a response to an increased local nitrate concentration depends on the plant's internal N status, the external supply of nitrate, and seem to be coupled with the auxin response pathway (Forde, 2002). But in another experiment the results suggest that auxin is not required for the response to a nitrate rich patch (Linkohr *et al.*, 2002). If the response is systemic, the locally increased nitrate concentration has to be recognized in the shoot. Then the shoot sends a signal to the roots which proliferate (Forde & Lorenzo, 2001). The nature of the signal send from shoot to roots is unknown (Walch-Liu *et al.*, 2006).

For different plants, such as maize or wheat, it is shown that local enhanced concentrations of nitrate, phosphate and potassium cause higher root respiration rates (Granato & Raper, 1989; Thoms, 1992; Sattelmacher & Thoms, 1995; vanVuuren *et al.*, 1996). The respiration rates are increased before and after new roots proliferate in the nutrient enriched patch.

Many experiments have dealt with the problems of nutrient deprivation for plants. Plants with an insufficient nutrient supply for example have a reduced shoot/root ratio (e.g. caused by N

deficiency), reduced net photosynthesis (e.g. caused by K deficiency) or earlier leaf senescence (e.g. caused by N or P deficiency) (Shuman, 1994; Marschner, 1995b). Generally, nutrient deprivation affects the plant on the morphological as well as on the physiological level.

A lot of different experimental setups have been used in order to imitate nutrient heterogeneities in laboratory experiments. In experiments, where nutrient solution is used (e.g. Drew, 1975a; Granato & Raper, 1989; Schortemeyer & Feil, 1996), the nutrient concentration is constant. But a small plant at the beginning of the experiment gets the same concentration as a bigger plant at the end of the experiment although the demand of nutrients is different. If the nutrient heterogeneity is made with compost (e.g. Wijesinghe & Hutchings, 1997; Wijesinghe *et al.*, 2005), different soils (e.g. Anghinoni & Barber, 1980), organic matter (e.g. vanVuuren *et al.*, 1996) or fertilizer (e.g. Rajaniemi & Reynolds, 2004), the nutrient concentration is not constant, because of nutrient uptake by plant and leaching by watering. In these cases the plant can have an oversupply of nutrients at the beginning and a lack at the end of the experiment. Furthermore, nutrient heterogeneities made with compost, soils or organic matter are composed of nutrients, soil organisms, different water content and different soil structure. In such experiments it is not clear to what factors plants respond to.

Nutrient patterns are formed in different ways e.g. as chequerboard pattern (Casper & Cahill, 1996; Day *et al.*, 2003a; Day *et al.*, 2003b), stripes (Anghinoni & Barber, 1980), patches (Nobbe, 1862) or as a result of 3*3 factorial design (Lamb *et al.*, 2004). In these experiments it is not clear, if pattern or nutrient concentrations are responsible for plant reaction.

Although there has been a lot of research about the impact of nutrient heterogeneity on plants, there are still gaps of knowledge. Mostly, the impact of nutrient heterogeneity on structural and functional responses of roots has been investigated. Therefore not only the structural and functional responses on heterogeneously distributed nutrients of roots but also of shoots, leaves and the whole plant have to be measured. Furthermore it is unknown whether plants with an insufficient nutrient supply respond as well fertilised or at what level of nutrient heterogeneity they respond to nutrient heterogeneity. In order to close these gaps of knowledge, a new experimental setup and design have been developed, where only nutrients were heterogeneously distributed.

1.3 Hypotheses and Questions

The following assumptions are made in this investigation: plants only differ in nutrient supply (homogeneous, heterogeneous), but the total amount of supplied nutrients is equal. Plants with a heterogeneous nutrient supply have bigger root systems on those root sides, which got a higher concentration of nutrients. This response has often been shown in the literature before (see 1.2 p. 2). The plants get more nutrients from the bigger root system but have an additional investment of carbon for building and keeping it alive. Two possibilities result from this effect. First, if plants are limited in nutrients and the distribution of nutrients is heterogeneous, a higher nutrient uptake by a bigger root system would cause a higher above ground biomass in comparison with plants which get a homogeneous supply of nutrients. On the other hand, if investment of carbon to the roots is higher than the gain of nutrients, an unequal supply of nutrients would produce a smaller above ground biomass.

Therefore, following hypotheses and questions arise:

1. A limited and heterogeneous supply of nutrients causes a higher above ground biomass compared to plants with a limited and homogeneous nutrient supply.
 - Does the nutrient status of plants have an impact on the response to nutrient heterogeneities?

It has not been shown how big the contrast between high and low nutrient areas has to be before plants first respond to nutrient heterogeneity.

- At what level of nutrient heterogeneity do plants respond?
2. Plants respond to nutrient heterogeneity with a structural (root proliferation, above ground biomass) and a functional (more nutrients, higher investment of C in the roots) change.
 - But do plants really respond structurally and functionally to nutrient heterogeneity?
 3. Nutrient heterogeneities have an effect on the whole plant.
 - But do nutrient heterogeneities really affect the whole plant or are there only localized effects?

2. Materials and Methods

2.1 Plant material

For the experiments *Zea mays* hybrid Helix was used. *Zea mays* (order: poales, family: poaceae), an important agricultural plant, is a monocotyledonous, annual plant with C_4 photosynthesis and a fibrous root system.

For homogeneity of plant material 120 plants were germinated for each experiment in sand-filled pots with a volume of 221 cm³ and were watered with tap water. 15 days after germination plants were cleaned from the sand with tap water and weighed. 45 plants, with nearly equal weights were used in the experiment. All plants had 4 leaves and 6 to 8 roots at the start of the experiment. If a plant had an uneven number of roots, one root was cut off. By this procedure it was possible to position the same number of roots in each side of the split root pots. Each experiment lasted 4 weeks.

2.2 Growth Conditions

Plants were grown in a laboratory with a mean humidity of 31.8 ± 6.5 (r.h.) and temperature of 21.1 ± 1.3 (°C) during the experimental period. As light source, lamps (OSRAM FLUORA 36/77) were used. Photon flux density averaged $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Day length was 12 h.

In the experiments natural quartz sand (SiO_2 : 95.7 weight-%) was used in order to minimize the exchange and adsorption of nutrients by substrate. The field capacity was 6.8 ± 0.5 %. The sand grain size distribution was: 0 – 63 μm 0.27 %; 63 – 200 μm 8.2 %; 200 – 630 μm 86.7 %; 630 – 2 mm 3.94 %. Sand was washed with tap water before use and filled in split root pots. Then the plants were placed in the split root pots.

2.3 Split root pots

A split root system was used. It provides the simplest case of spatial heterogeneity, with only 2 regions.

The split root pots were made out of two equal flower pots (each had a volume of 2 l), which were glued together. At the upper part a PVC boundary was attached. To close the flower pots at the bottom, sieve cloth was fixed. The mesh size of the sieve cloth (pore size: 250 μm ; polyamide; THOMAPOR) was big enough that water or nutrient solution could drain out of the sand filled flower pots but maize roots were not able to grow through. This was tested in several preliminary experiments. The total volume of one split root pot was 5464 cm³. The volume, which was filled with sand, was about 4250 cm³.

Flower pots and the boundary from the split root pots were glued with a hot-melt adhesive (HENKEL). In a preliminary test it was proved that the hot-melt adhesive did not release plant toxic ions. These chemical analyses (ICP-OES) were done in the Central Division of

Analytical Chemistry (ZCH) in the Research Centre Juelich. For the multi-element determination ICP-OES (Inductively Coupled Plasma with Optical Emission Spectroscopy) was used. The liquid sample was introduced into the inductively generated argon plasma through a nebulizer system and excited. The spectrum emitted was transferred into a spectrometer (TJA-IRIS) where it was decomposed into the individual wavelengths and evaluated. The intensities of the spectral lines were measured by CID semiconductor detectors. Calibration was effected by multi-element solutions mixed from standard solutions.

2.4 Irrigation system

Sand culture allowed using nutrient solution for producing nutrient heterogeneity. A special irrigation system was developed for this purpose.

The system to add nutrient solution to the split root pots was built from parts of a commercial irrigation system (NETAFIM). Pressure controlled dripper seeped a known value ($0.57 \pm 0.01 \text{ ml} \cdot \text{s}^{-1}$) of nutrient solution per time in the split root pots. In a preliminary experiment all drippers in the system were tested. Drippers, which seeped nearly the same amount of solution, were set as a pair in the irrigation system, one on the left side, the other on the right side of the split root pot. Therefore the plants got the same amount of solution per time on both sides (Figure 1). Every plant received 114 ml (57 ml per side) nutrient solution per day. Immersion pumps (BARWIG) were used to pump the nutrient solution in the irrigation system.



Figure 1: Irrigation system with maize plants in split root pots.

One hour before the nutrient solution was added, potentially remaining residues of nutrients present in the split root pots from the previous irrigation were rinsed out of the sand with 1.3 l demineralised water. The amount of demineralised water which was necessary to rinse out

the remaining nutrient solution was determined in a preliminary test. The osmolarity of the run off solution was detected with an osmometer (FISKE MICRO-OSMOMETER MODEL 210).

2.5 Nutrient solution

2.5.1 Steady-state nutrition and relative growth rate (RGR)

Traditionally, constant amounts of nutrients are given to plants independent of their nutrient demand. In that case a growing plant could experience a situation of nutrient deprivation when fewer nutrients are delivered compared to demand.

An alternative option is to adjust the given amount of nutrients to development of the plant. This steady-state nutrition allows to control plant development with the amount of nutrients given to the plants and to investigate the impact of the nutrient status of plants on the respond to nutrient heterogeneities.

In steady-state nutrition the amount of nutrients is related to the increase of biomass per time. This approach aims for a constant internal concentration of nutrients in the plants. From this it follows that the internal concentration of nutrients in the plants stays constant (Ingestad & Agren, 1992). Therefore, the amount of nitrogen in the different internal pools remains relatively constant (Imsande & Touraine, 1994). The increase of the amount of nitrogen in the plant is proportional to the increase in biomass.

This can be described with (1):

$$d(n/W)dt = 0 \quad (1)$$

With:

n = amount of nutrient in the plant

W = biomass of the plant

From this it follows that:

$$\frac{1}{n} * \frac{dn}{dt} = \frac{1}{W} * \frac{dW}{dt}$$

or

$$RAR = RUR = RGR \quad (2)$$

With:

RAR = relative addition rate

RUR = relative uptake rate

RGR = relative growth rate

or

$$\begin{aligned} \text{RGR} &= \frac{1}{W} * \frac{dW}{dt} = \frac{d(\ln W)}{t_2 - t_1} \\ \text{RGR} &= \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \\ \text{RGR} &= \frac{\ln(W_2 - W_1)}{t_2 - t_1} \\ \text{RGR} * (t_2 - t_1) &= \ln(W_2 - W_1) \\ \frac{W_2}{W_1} &= e^{\text{RGR} * (t_2 - t_1)} \\ W_2 &= W_1 * e^{\text{RGR} * (t_2 - t_1)} \end{aligned} \quad (3)$$

With:

W_1, W_2 = biomass of the plant at different times [g]

t_1, t_2 = time

In all experiments the nutrient supply was adapted to the relative growth rate (RGR). So it was possible to grow the plants continuously and constantly with limiting ($0.5 * \text{RGR}$) or with excess ($1.5 * \text{RGR}$) supply of nutrients. The maximum RGR and the average of nitrogen content of the plants were determined for the specific growing condition in a preliminary experiment.

2.5.2 Experiment for determination of the RGR of maize

Maize plants were grown in sand in a greenhouse. The plants were fertilised with 120 ml Hoagland solution per day which complied with an optimal nutrition. Twice a week 5 plants were harvested. After roots and shoots were weighed, the plants were dried at 75°C . Then dry weight was determined (Figure 2) and the RGR (3) calculated. From day 15 to 26 the RGR was on average 0.22 and 0.04 from day 29 to 40 (Figure 3). The means of these two periods were used for calculating the nutrient supply in the experiments.

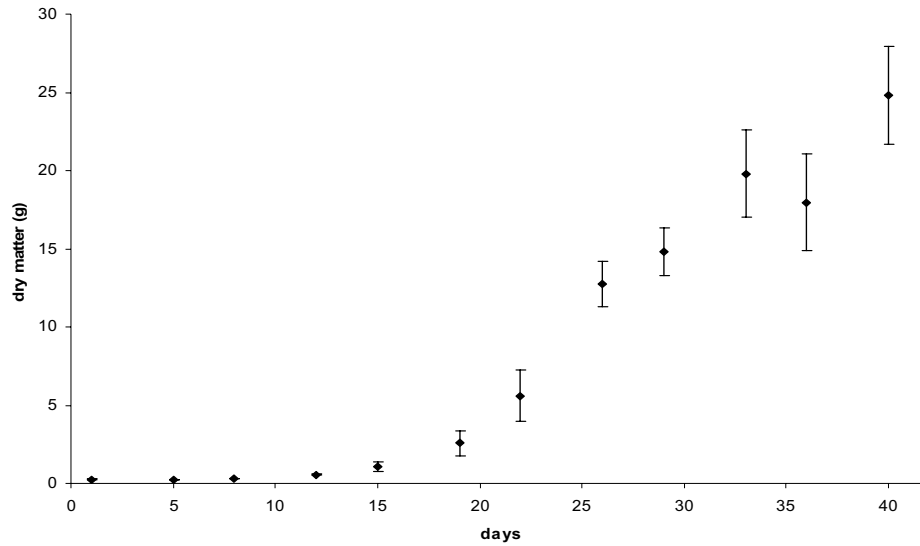


Figure 2: Dry matter of maize plants in the preliminary experiment. Means and standard deviation are shown. (n = 5)

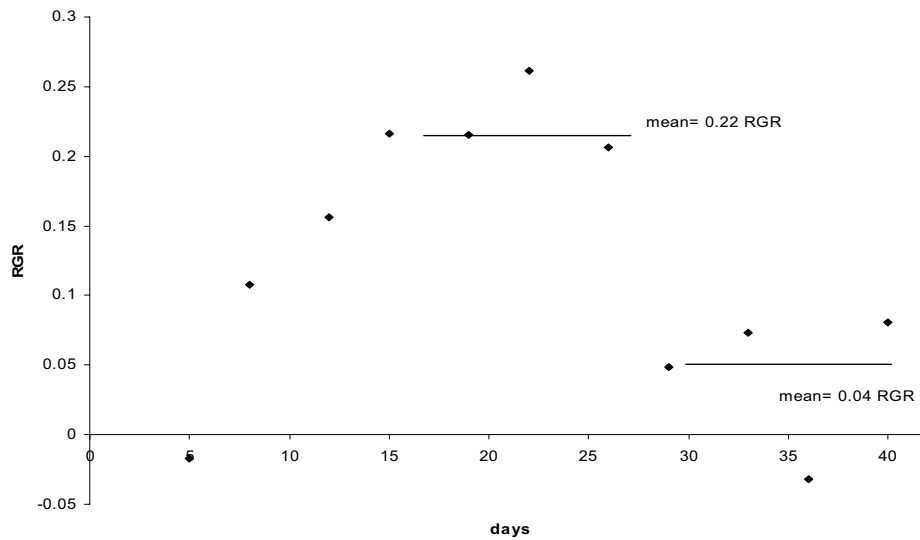


Figure 3: RGR of maize in the preliminary experiment. The two lines show the average of the RGR in this time period.

After each harvest the dry plants were ground in a ball mill and nitrogen content was analysed with CHNS ANALYSATOR. The sample (5 mg) was burned at a temperature of 1000 °C in flowing oxygen for C, H, N analysis in the analyser (Leco CHNS-932). The CO₂, H₂O, NO_x and SO₂ combustion gases were passed through a reduction tube with helium as

the carrier gas for converting the NO_x nitrogen oxides into N₂ and binding the free oxygen. The CO₂, H₂O and SO₂ combustion gases were measured by selective IR detectors. After corresponding absorption of these gases, the content of the remaining nitrogen was determined by thermal conductivity detection. The nitrogen content was on average 2.5 % of dry biomass of the plants.

Two experiments were run. In one setup plants were grown under limiting (0.5*RGR) and in the other under high nutrient conditions (1.5*RGR). The treatments of the nutrient supply adapted to 0.5*RGR and 1.5*RGR were selected, because these nutrition status were extreme but still allowed the plants to survive and to grow. If there was an impact of the nutrient status of plants on the response to nutrient heterogeneity, then it would be detectable.

In the experiment where the nutrient supply was adapted to 0.5*RGR, the half of the calculated RGR and the average nitrogen content from the preliminary experiment was used to generate the amount of nitrogen supply. In the other experiment the nutrient supply was adapted to 1.5*RGR. Every second day nutrient solution was changed.

For these two experiments the N demand of the plants was calculated as followed (4):

$$N(\%)_{Fm\ Plant} = \frac{N(\%)_{Dm}}{k}$$

$$N_{Fm\ Plant} = \frac{Fm_{Plant}}{100} * N(\%)_{Fm\ Plant} \quad (4)$$

With:

$N(\%)_{Dm}$ = proportional amount of nitrogen in plant dry matter [%]

k = ratio of plant fresh/dry matter

$N(\%)_{Fm\ Plant}$ = proportional amount of nitrogen in plant fresh matter [%]

Fm_{Plant} = plant fresh matter [g]

$N_{Fm\ Plant}$ = nitrogen content in plant fresh matter [g]

With formula 3 the theoretical increase of biomass in a period could be calculated. For this biomass the nitrogen content of the fresh matter was determined (4). The nitrogen demand of the plants in a time interval (2 days) was determined as followed (5):

$$N_{Fm Plant t_1 - t_2} = N_{Fm Plant t_2} - N_{Fm Plant t_1}$$

$$N_{per day} = \frac{N_{Fm Plant t_1 - t_2}}{(d/14.0067)} \quad (5)$$

With:

$N_{Fm Plant t_1 - t_2}$ = nitrogen demand in a time interval [g]

d = days

14.0067 = molar weight of nitrogen [g/mol]

$N_{per day}$ = nitrogen demand of the plant per day [mol]

The calculated amount of nutrients was added with the irrigation water. A nutrient solution was used, adapted to a Hoagland nutrient solution (Hoagland & Arnon, 1938). Nitrogen sources were KNO_3 and $Ca(NO_3)_2$. Only nitrate was used as nitrogen source in order to avoid possible differences in the response caused by different nitrogen forms (e.g. ammonium). All other nutrients were in constant ratios to nitrogen (Table 1) according to Hoagland & Arnon (1938).

Table 1: Components of the Hoagland solution and the relationships of KNO_3 and $Ca(NO_3)_2$ to the other nutrients.

	Nutrients	Relationship of the nutrients
Macronutrients	KNO_3	1
	$Ca(NO_3)_2$	1
	$MgSO_4$	0.4
	KH_2PO_4	0.2
Micronutrients	$MnCl_2$, $CuSO_4$, $ZnSO_4$, H_3BO_3 , Na_2MoO_4	0.2
	Fe-EDTA	0.2

All plants received equal total amounts of nutrients, but with different fractions supplied to each of the two root compartments (Figure 4). The treatment 0.5/0.5 was the homogeneous control, where both root sides received the same amount of nutrients. The other 5 treatments established unequal distribution of nutrients for the two root sides ranging from 0.6/0.4, 0.7/0.3, 0.8/0.2, 0.9/0.1 up to 1.0/0.0 (heterogeneous treatment).

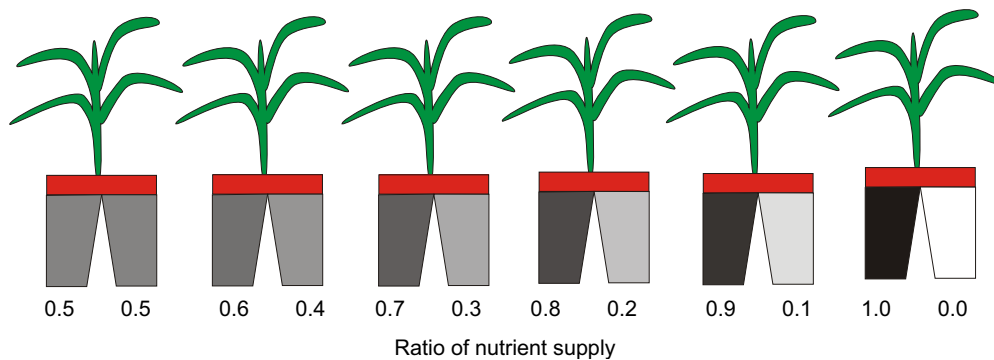


Figure 4: Schematic drawing of the 6 different treatments. Heterogeneity is increasing in the treatments.

All 6 different treatments (Figure 4) were carried out with 9 replicates in each experiment. Because of logistical reasons only 5 treatments per experiment could be done simultaneously. The additional treatment (0.6/0.4) was done afterwards and only biomass was measured.

In the experiments nutrients were unequally supplied to plants spatially. During the experiments this kind of supply was not varying. For this spatial heterogeneity the simplest case of patchiness was used. The nutrients were supplied in only two different regions. Plants perceived and responded to this unequal supply of nutrients.

For a better understanding of the further work some terms had to be defined:

- Nutrient heterogeneity = functional and spatial heterogeneity of nutrients which was constant over time
- High nutrition experiment = experiment in which the nutrient supply was adapted to $1.5 \cdot \text{RGR}$
- Low nutrition experiment = experiment in which the nutrient supply was adapted to $0.5 \cdot \text{RGR}$

2.6 Harvest of the plants

The plants were harvested 29 days after the experiment had started. The total age of the plants was 44 days. Plants from different treatments were harvested at nearly the same day time in order to minimize differences in sugar and starch content (see 2.7.2.2.3, p. 17; 2.7.2.2.4, p. 19) due to diurnal courses of carbohydrates.

2.7 Structural and functional Parameters

Structural and functional parameters were analyzed to characterize the response of plants to heterogeneous nutrient availability.

2.7.1 Structural Parameters

2.7.1.1 Development of the plants

During the experimental period the development of the plants was observed in order to recognize if plants respond to nutrient heterogeneity with a change of development. Twice a week quantity and actual state of the leaves were classified in 4 different states (Figure 5) and reported.

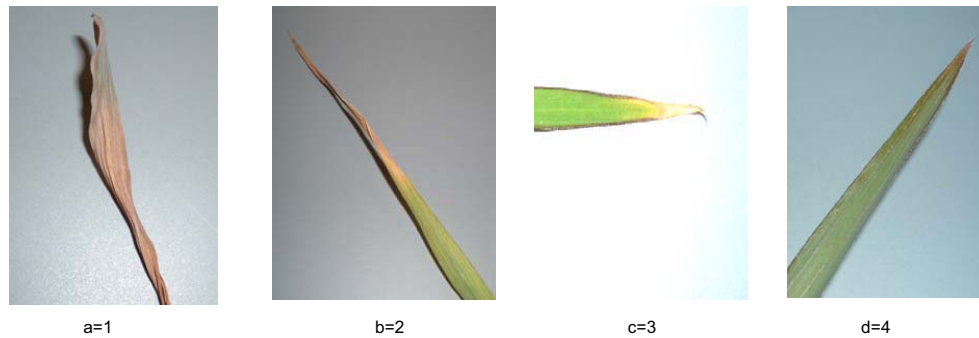


Figure 5: 4 states of the leaves: a) dead leaf, b) leaf with a dry tip followed by a yellow part which is ending in the light green leaf, c) green leaf with a small dry tip, d) green leaf

The 4 different classes got a number from 1 to 4 in order to describe the development of the treatments statistically. The average actual state of leaves (AASL) in the different treatments was calculated according to (6):

$$AASL = \frac{\sum ASL}{\sum L} \quad (6)$$

With:

AASL = average actual state of leaves

ASL = actual state of the different leaves of the plants in the respective treatment

L = number of leaves of the plants in the respective treatment

2.7.1.2 Biomass

At harvest the shoots were cut off with a razor blade immediately above the roots and the weight of fresh matter was determined. The roots in the two root compartments were cut with a razor blade in the middle of the split root pot. This allowed harvesting the roots for every root compartment separately. Sand was rinsed from the roots with tap water. Carefully, attached droplets were removed by a paper tissue afterwards. The fresh mass was measured. Roots and shoots were dried in an oven at 75°C for 14 days until weight constancy and dry matter were determined. The required drying time was tested in a

preliminary experiment. The dry weights were used for statistical analyses. Plant total dry matter, total root dry matter and shoot/root ratio were calculated.

2.7.1.3 Root structure

A main response of plants to nutrient heterogeneity reported in previous analyses (see 1.2; p. 2) was the proliferation of roots within the nutrient patch. Therefore, not only total root biomass but possible change of root surface, length and volume were of interest.

After harvesting, fresh roots of 3 plants per treatment were taken for analyzing the root structure. This was done at rinsed roots with a special scanner system and the software WINRHIZO (Régent Instruments Inc.). Because of two light sources, the scanner system had an optimal illumination of the objects. One lamp was below the scanner glass and one in the scanner cover. Analyzed parameters were total root length, root volume and root surface. After analyzing, the roots were dried and dry matter was determined.

Furthermore the specific root length was calculated (7).

$$SRL = \frac{RL}{TRDM} \quad (7)$$

With:

SRL = specific root length [m/g]

RL = root length [m]

TRDM = total root dry matter [g]

2.7.1.4 Leaf area

The determination of plant development was qualitative. For quantification the leaf area was measured. For 4 plants of each treatment the leaves were cut off the shoot with a razor blade during harvest. These leaves were put on a paper and photocopied. The copied leaves were cut out and weighed. With the mass of the copies of the leaves and the calibration of area to mass of the used copy paper, the leaf area could be calculated.

2.7.2 Functional Parameters

2.7.2.1 Photosynthesis

The first step in primary metabolism is photosynthesis. Photosynthesis can be influenced by N limitation (Lu & Zhang, 2000). Therefore the rate of photosynthesis at different treatments was measured in order to detect potential limitation of photosynthesis.

The rate of photosynthesis was measured twice a week with the portable photosynthesis measurement system LI 6400 (LI-COR BIOSCIENCES INC.). Photosynthesis was measured from five plants per treatment at the second leaf from the top of the plant. The system measured the gas exchange from the attached leaf every 5th second for 5 minutes. CO₂

concentration in the reference cuvette was 400 μmol and the air temperature was 22°C. Light conditions in the cuvette were adapted to the experimental light conditions. Photon flux density was 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The photosynthesis rate was calculated by the software of the system (LI-COR, 2005) .

2.7.2.2 *Metabolites*

During the harvest the second and third leaves from top of the plant were weighted and frozen in liquid nitrogen. These samples were used for analyzing the chlorophyll, sugar, starch and free amino acid content. This was done with 4 plants per treatment. For each of these 4 plants 200 - 300 mg of the root tips (the first 1.5 cm from tip to base) from each root compartment were cut off in order to measure the sugar and starch content.

2.7.2.2.1 *Extraction of chlorophyll and carbohydrate*

For analyzing the chlorophyll and soluble carbohydrate content in leaves and the sugar content in the roots an ethanol/water extraction was used. First, every frozen leaf was extracted for 15 minutes with 800 μl 80 % ethanol/water (80:20 v/v, 10 mM HEPES) at 80°C. The supernatant was transferred in a reaction tube and stored on ice. Then the leaves were extracted a second time with 800 μl 50 % ethanol/water (50:30:20 v/v/v, ethanol, 10 mM HEPES, desalted water) for 15 minutes at 80°C. Again the supernatant was given in the reaction tubes which were stored on ice. After that a third extraction step was made, where the leaves were extracted with 400 μl 80 % ethanol/water (80:20 v/v, 10 mM HEPES). If the leaves were not pale after the third extraction, the extraction was repeated with 400 μl 80:20 ethanol/water at 80°C. At the end the combined supernatants were filled to a unique volume with ethanol (80 %). The same procedure was done with the root tips for carbohydrate analysis.

The pale and extracted plant material was grinded to analyze the starch content. A stainless steel ball (diameter 7 mm) was used in a mixer mill (RETSCH, MM200). In 300 μl distilled water the plant material was grinded. With additional 200 μl distilled water the ball was washed. The samples were autoclaved for 99 minutes at 121°C and 1.2 bar in order to destroy the plastid membranes. After extraction the samples were stored at -20°C until further analysis were done.

2.7.2.2.2 Chlorophyll

The actual states of leaves during the experiment were described. The investigation of the chlorophyll content at the end of the experiment completed this parameter.

The chlorophyll concentration of the leaves was measured directly after extraction according to Arnon (1949). First, the samples were mixed and then 400 µl of the extract was diluted with 400 µl 80 % ethanol. Then the samples were centrifuged for 5 minutes at 14,000 rpm. The supernatant was analysed in a photometer at $\lambda = 652$ nm against 80 % ethanol/water (80:20 v/v, 10 mM HEPES). The chlorophyll concentration was calculated from the optical density (8):

$$C_{\text{chl}} = \frac{\text{OD} * V_{\text{cuvette}} * \frac{V_{\text{extract}}}{V_{\text{aliquot}}}}{34.4 * m_{\text{FW}}} \quad (8)$$

With:

C_{chl} = chlorophyll concentration [$\text{mg} \cdot \text{g}^{-1} \text{FW}$]

OD = optical density

V_{extract} = volume of the extract [μl]

V_{aliquot} = volume of the aliquot [μl]

V_{cuvette} = volume of the cuvette [μl]

34.4 = conversion factor for Chlorophyll [ml/mg]

m_{FW} = fresh weight of the plant material [mg]

2.7.2.2.3 Soluble carbohydrates

If plants responded with increasing biomass to rising heterogeneity, they already would have a benefit from the heterogeneous nutrient supply before biomass increased. This benefit could be detectable in a change of the sugar and starch concentration. Therefore it was necessary to measure an additional parameter of primary metabolism.

The content of soluble carbohydrates (glucose, fructose and sucrose) in leaves and roots was analyzed with a coupled enzyme assay according to Jones *et al.* (1977). The increase of optical density at $\lambda = 334$ nm of the reaction from NADP^+ to $\text{NADPH} + \text{H}^+$ was detected. The starting enzyme was glucose-6-phosphate dehydrogenase (Figure 6). Successively the enzymes hexokinase, phosphoglucosomerase and invertase were given to the extract. With all these enzymes glucose, fructose and sucrose were measured.

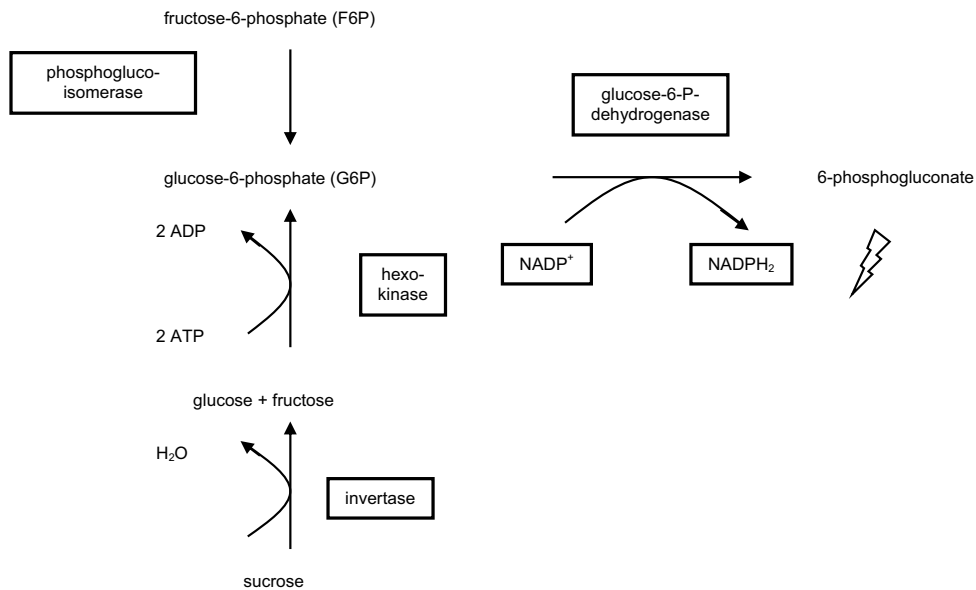


Figure 6: Schematic diagram of the coupled enzyme assay (Christ, 2005).

At 13,000 rpm for 4 minutes the enzymes glucose-6-phosphate dehydrogenase (5.6 U), hexokinase (6 U) and phosphoglucose isomerase (10 U) (ROCHE DIAGNOSTICS GmbH) were centrifuged. The pellet was dissolved in 200 μ l TRIS buffer (100 mM, 10 mM MgCl₂, pH 8.1). $\frac{1}{4}$ of a 1.5 ml reaction tube invertase (SIGMA-ALDRICH) was dissolved in 100 μ l TRIS buffer (ca. 5 U). A multiplate photometer (ht II ANTHOS MICROSYSTEME GmbH) was used for analysis. The master mix which was used for one 96-well-microtiter plate was made up of:

- 15.5 ml imidazol buffer (100 mM, 5 mM MgCl₂, pH 6.9)
- 480 μ l ATP (60 mg ml⁻¹)
- 480 μ l NADP⁺ (36 mg ml⁻¹)
- 200 μ l glucose-6-phosphate dehydrogenase

First 20 µl of the extract and then 160 µl master mix were given in the wells of the microtiter plate. After the first reaction finished, 2 µl of the next enzyme was given per well, successively (Figure 6). The soluble carbohydrate concentration was calculated with (9):

$$C_{\text{(hexose)}} = \frac{\Delta OD * \frac{V_{\text{(extrakt)}}}{V_{\text{(aliquot)}}}}{6.22 * 2.85 * m_{\text{FW}}} \quad (9)$$

With:

- C_{hexose} = carbohydrate concentration [$\mu\text{mol g}^{-1}$]
- V_{extract} = volume of the ethanolic extract [μl]
- V_{aliquot} = volume of the aliquot [μl]
- 6.22 = optical density of 1 $\mu\text{mol NADPH} + \text{H}^+$ ml^{-1}
- 2.85 = specific factor of the photometer [cm^{-2}]
- m_{FW} = fresh weight of the plant material [mg]

It had to be noticed that one molecule of glucose or fructose corresponded to one molecule of $\text{NADPH} + \text{H}^+$ but one molecule sucrose to two of $\text{NADPH} + \text{H}^+$. Therefore the sucrose concentration was calculated by dividing C_{hexose} by factor 2.

2.7.2.2.4 Starch

Nearly the same procedure as described in 2.7.2.2.3 (p. 17) was used to analyze the starch concentration. Instead of imidazol buffer TRIS buffer was used. The starch was enzymatic determined in glucose units (10).

$$C_{\text{starch(glucose)}} = \frac{\Delta OD * \frac{V_{\text{(inkub)}}}{V_{\text{(inkub - aliquot)}}} * \frac{V_{\text{(extract)}}}{V_{\text{(aliquot)}}}}{6.22 * 2.85 * m_{\text{FW}}} \quad (10)$$

With:

- $C_{\text{starch (glucose)}}$ = concentration of starch (glucose) [$\mu\text{mol} * \text{g}^{-1}$]
- V_{inkub} = incubation volume [μl]
- $V_{\text{inkub-aliquot}}$ = incubated extracted volume [μl]

Total non-structural carbohydrate (TNC) was the sum of glucose, fructose, sucrose and starch content or concentration.

2.7.2.2.5 Free amino acids

Nitrogen has a big impact on plant growth (see before). Free amino acids are one of the internal pools of N and C in the plant. In maize plants nitrate assimilation occurs in the leaves (Andrews, 1986). Therefore the free amino acid concentrations were measured in the leaves of the control and the 1.0/0.0 treatment. If there was a difference in N uptake it would be detectable in these treatments.

Frozen leaves (-80 °C) were grinded with liquid nitrogen in a cooled mortar. Then 70 - 130 mg of this material was mixed with 500 µl extraction solution (50 % v/v ethanol in 0.1 M HCl) and 50 µl internal standard (10 nmol norvalin). The material was grinded again and given in a cooled reaction tube. Remaining material in the mortar was rinsed with 500 µl extraction solution in the extraction tube. After that the homogenates were centrifuged (14000 rpm) in a cooled centrifuge (4 °C) for 15 minutes. The supernatants were given in new cooled reaction cups. The leaf extracts were analyzed using the EZ:faast physiological kit for GC-MS (PHENOMENEX, Torrance, CA, USA) designed for analyzing amino acids in complex sample matrices. Aliquots of 200 µl were prepared for amino acid analyses following the sample preparation protocol described in the EZ:faast AAA kit user manual. After extraction of the amino acid derivatives into organic phase the samples were dried over sodium sulfate for 1 h. Then they were filtered through glass wool. GC-MS analyses were performed on the Finnigan MAT GCQ GC-MS, and using XCalibur software (THERMO ELECTRON CORPORATION, Waltham, MA, USA). The GC column used was the ZB-AAA GC column (15 m x 0.25 mm ID) from Phenomenex. The injector temperature was 250 °C. Samples (1 µl) were injected in the split mode (1:10). The inlet pressure of helium as the carrier gas was set to 4 psi. The oven temperature was initially 110 °C, then programmed at 15 °C/min increase to 300 °C (held for 5 min). The transfer line and ion source temperatures were 275 °C and 175 °C, respectively. The mass range scanned was 50 – 650 u.

2.7.2.3 C, N and S content

In addition to the analyses of biomass, photosynthesis, sugar and starch the total C content had to be detected. Furthermore N and S contents were measured. If plants had a higher nutrient uptake caused by root proliferation in the nutrient patch this would be possible to detect in roots and shoots.

The analyses of carbon, nitrogen and sulphur were done with dry plant material. Shoots of each treatment were pooled and grinded in a ball mill. From this material 4 samples were taken for analysis. For analysis of the C, N and S content of the roots three complete root systems of the left and right sides were pooled and grinded. As before, 4 samples were analysed. Analysis of C, N and S was done with a CHNS-Analysator (LECO CHNS-932).

2.8 Statistics

For the statistical analyses of the biomass only the weights of dry matter were used. Analyses were done with the statistic software Sigma Stat 2.03. In order to describe the data, mean, standard deviation and box whisker plots were used. In box whisker plots, data are described by the 10, 25, 50 (median), 75 and 90 percentiles.

To compare only two different treatments or variables a t-test was done. If there were more than two treatments or variables a ONE-WAY ANOVA ($p = 0.05$) was calculated. For this it was necessary that the data were normally distributed and had equal variances. If this was not the case, a non parametric test (Kruskal- Wallis ANOVA on ranks; $p = 0.05$) was used. If there were significant differences between the tested groups a pair wise multiple comparison was done afterwards with the Tukey Test ($p < 0.05$).

To get general information of the impact of nutrient heterogeneity and nutrient status on plants, parameters were classified in two groups (impact; no impact). The percentages of these two groups were calculated.

3. Results

The results were divided into 3 parts: structural, functional and the summary of structural and functional parameters. The summary is necessary because responses of structure and function are often mutually dependent.

For answering the question if the nutrient status of plants had an impact on the reaction of plants to nutrient heterogeneities, the results of the parameters at high and low nutrient conditions were compared.

3.1 Structural parameters

3.1.1 Development of the plants in the experiments: structural measurements

3.1.1.1 *Number and average actual state of the leaves*

During the experiment the development of the plants was observed. At the beginning of the experiments all plants had 4 green leaves. During the experimental period new leaves were formed (Figure 7a, b).

At the high nutrition experiment the average number of leaves was increased from 4 to 7.4 (Figure 7a), but the treatments did not statistically significant differ. The average actual state of the leaves was reduced from 4 to 3.7 (Figure 7c). A statistical significant difference between the treatments 0.8/0.2 and 1.0/0.0 at the marked day (day 15) was found.

At the low nutrition experiment the average number of leaves was increasing from 4 to 6.7 (Figure 7b). The average actual state of the leaves was reduced in all treatments (Figure 7d). There were no differences in the development of the above ground biomass of the plants between the treatments and the average numbers of leaves did not differ, except at the marked day. There was a statistical significant difference between the control (0.5/0.5) and the treatment 1.0/0.0. The average actual state of the leaves was reduced from 4 to 2.8 during the experimental period.

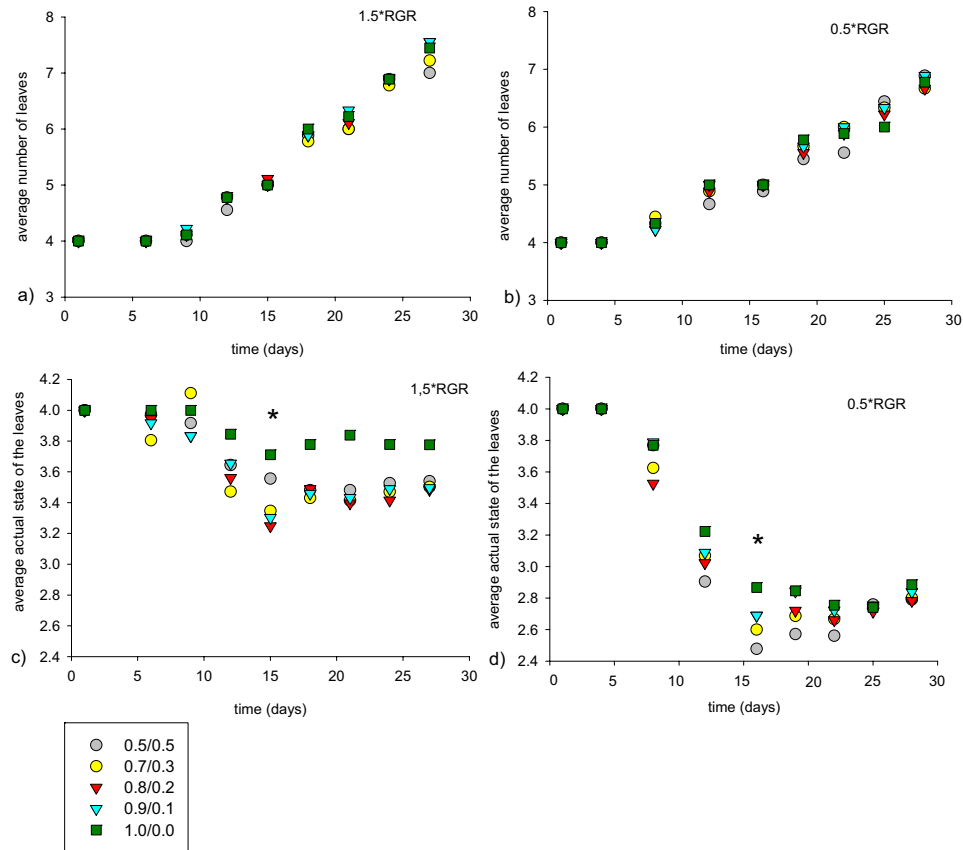


Figure 7: Comparison of leaf development (a, b) and average actual state of leaves (c, d) after transplantation to the split root pots. Means are shown in the graphics. Different symbols indicate the treatments. Significant differences were marked with *. Statistical analyses were done by a ONE-WAY ANOVA ($n=9$; $p<0.05$) or if normality test failed a Kruskal-Wallis One Way Analysis of Variances on Ranks ($n=9$; $p<0.05$) was used.

The number of leaves increased in both experiments. After day 20, plants with a higher nutrition began to develop more leaves than plants with a lack of nutrients (0.5*RGR experiment).

At both nutrient conditions the average actual state of the leaves was reduced in all treatments. During the first 10 days the state of the leaves was nearly the same. Afterwards, in the high nutrition experiment more leaves were green and healthy until the end of the experimental period than for the plants with a lack of nutrients. In both experiments only once a statistical significant difference was existent.

Furthermore, no impact of nutrient heterogeneity in the development of the above ground biomass could be detected in the experiments.

3.1.1.2 Leaf area

At the end of the experiments the leaf area was measured. Leaf area of the treatments in the respective experiments did not differ significantly (Figure 8). The nutrient heterogeneity did not affect leaf area. But in the experiment with 1.5*RGR the leaf area was much higher (on average 250 cm²) (Figure 8a) than in the experiment with a lack of nutrients (Figure 8b) (on average 130 cm²).

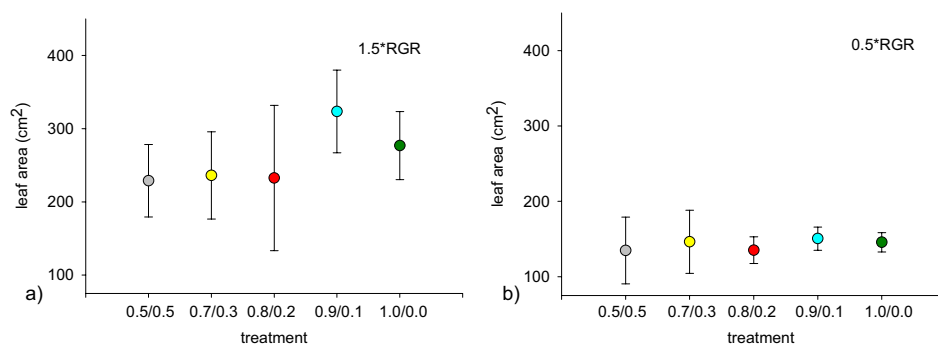


Figure 8: Leaf area of plants with a high or low nutritional status. The leaf area was measured of four plants per treatment. Means and standard deviations are shown in the graphics. Statistical analyses were done by ONE-WAY-ANOVA (n=4; p<0.05). There are no statistically significant differences between the treatments.

3.1.3 Biomass

3.1.3.1 Treatments 0.6/0.4

The treatments 0.6/0.4 of the experiments were done in subsequent experiments, because of logistical reasons in the experimental setup. Therefore the 0.6/0.4 treatments were run at other seasons than the respective experiments. These plants started with the same weight and got the same amount of nutrients as the plants in the 1.5*RGR experiment or in the 0.5*RGR experiment before. At the end of the experiment these plants got higher biomass (Figure 9 - 13), but showed equal shoot/root ratios (Figure 14) compared with all other treatments of the respective experiments. Therefore the data of the 0.6/0.4 could be used in the analysis.

3.1.3.2 Total dry matter

Total dry matter is the sum of shoot and root dry biomass. In the experiment with the nutrient status adapted to 1.5*RGR the biomass were quite uniform (Figure 9a). Only the treatment 0.6/0.4 was significant different (see 3.1.3.1, p. 24). In the experiment with low nutrient addition (0.5*RGR) the total dry matter did not differ between the treatments (Figure 9b).

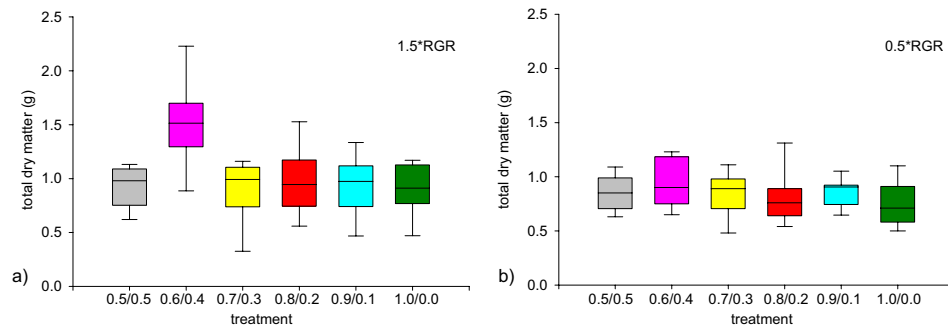


Figure 9: Comparison of the total biomass of plants grown at high and low nutrient conditions. Treatments 0.6/0.4 were done afterwards and could not be compared with the other treatments of the experiment. Statistical analyses were done by a ONE-WAY-ANOVA ($n=9$; $p<0.05$).

For both experiments the total biomass were nearly the same. An unequal distribution of nutrients had no impact on the total biomass, but different level of nutrition caused differences in the total biomass.

3.1.3.3 Shoot biomass

The experiment with a supply of high amounts of nutrients generated equal shoot biomass except for the 0.6/0.4 treatment, which was done in a subsequent experiment (Figure 10a). The shoot biomass were on average 0.62 ± 0.2 g (0.6/0.4 not included).

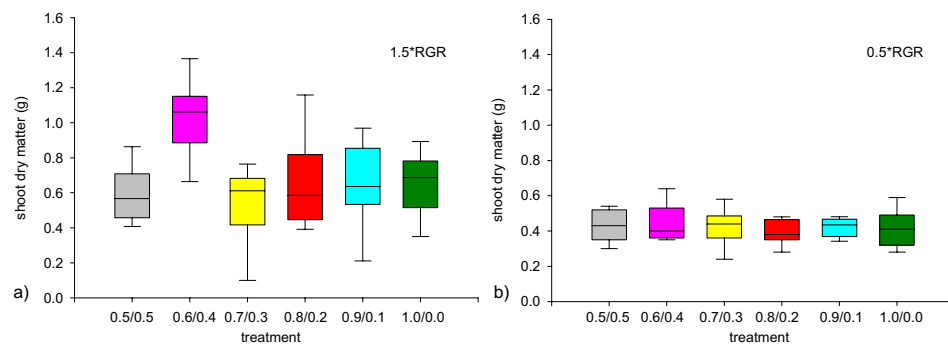


Figure 10: Comparison of shoot biomass of plants grown at high and low nutrient conditions. Treatments 0.6/0.4 were done afterwards and could not be compared with the other treatments of the experiment. Statistical analyses were done by a ONE-WAY-ANOVA ($n=9$; $p<0.05$).

In the experiment with a nutrient supply adapted to 0.5*RGR shoot biomass were not influenced by nutrient heterogeneity (Figure 10b). On average the biomass were about 0.42 ± 0.08 g.

In addition in both experiments an unequal distribution of nutrients had no impact on the shoot biomass. The comparison of the shoot biomass showed that the experiment with a high nutrient level had higher shoot biomass than the experiment with a low amount of nutrients.

3.1.3.4 Root biomass

Total root dry matters were calculated as the sum of biomass of the left and the right root sides. When the nutrient addition was adapted to 1.5*RGR, the total root biomass were similar in the different treatments (except for treatment 0.6/0.4) (Figure 11a). Total root dry matters were on average 0.26 ± 0.1 g (treatment 0.6/0.4 not included).

A low amount of nutrients caused nearly the same total root matters in the different treatments (Figure 11b). The weight was on average 0.39 ± 0.11 g.

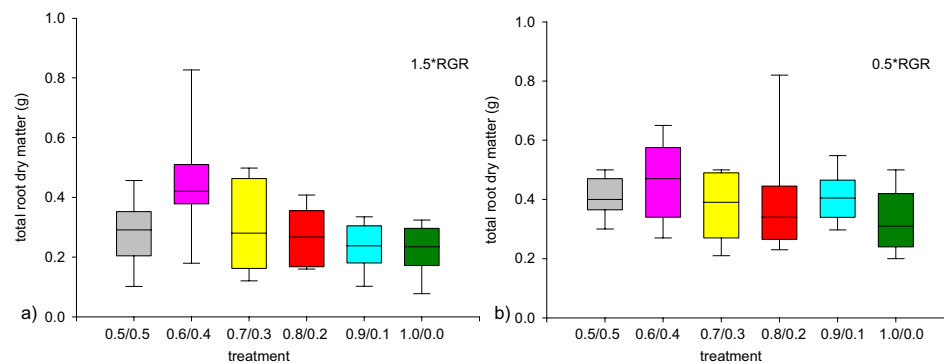


Figure 11: Comparison of the total root biomass of plants grown at high and low nutrient conditions. Treatments 0.6/0.4 were done afterwards and could not be compared with the other treatments of the experiment. Statistical analyses were done by ONE-WAY-ANOVA ($n=9$; $p<0.05$).

Nutrient heterogeneity did not affect different total root biomass. But the nutrient levels had an impact on the total root biomass. On average the total root biomass was lower in the high than in the low nutrition experiment but were not statistically significant different.

The comparison of the root dry matters in the different split root sides showed at the 1.5*RGR experiment (Figure 12a) that an equal distribution of nutrients (control treatment 0.5/0.5) caused equal root biomass. In the other treatments the root dry matters of the split

root sides were statistically significant different within the treatments. Root biomass were higher at the split root sides which got a higher concentrated nutrient solution. It was striking, that the increase in nutrient solution concentrations at the left root sides created no increased biomass. At the right root sides the concentration of the nutrient solution decreased, but the biomass did not differ (comparison done without 0.6/0.4 treatment).

If plants were grown under low nutrient conditions (0.5*RGR) the nutrient supply generated equal root biomass for the split root sides in the control as well as in the 0.6/0.4 treatment (Figure 12b), but the root dry matters of all other treatments were different. As before in the 1.5*RGR experiment, at the left root sides which got a higher concentrated nutrient solution the root dry matters were higher compared to the respective right split root sides. Although the concentration of the nutrient solution was gradually increased respectively decreased in these treatments, the root biomass of the left and the right root sides did not reflect this.

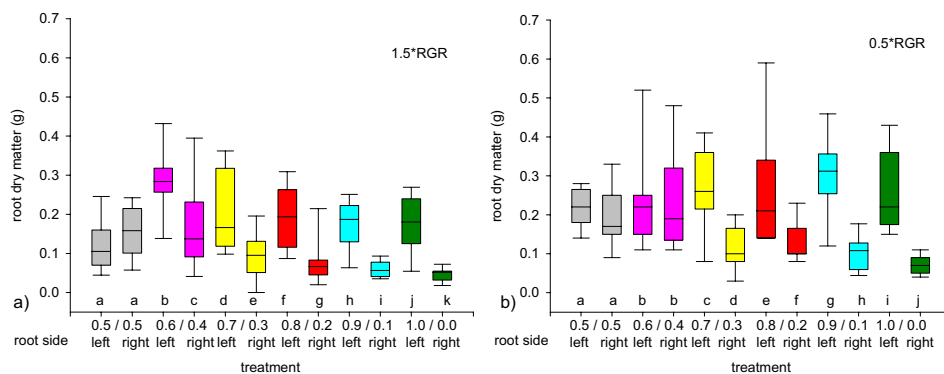


Figure 12: Comparison of the root biomass of the left and the right root sides in the different treatments at high and low nutrient conditions. Different letters mark statistical significant differences within the treatments. Statistical analyses were done by t-test (n=9; p<0.05).

In both experiments different total amounts of nutrients were supplied to the different split root sides during the experimental period. Though the total amounts of supplied nutrients were different in the experiments the root dry matters of the respective split root side were equal in both experiments (Figure 13).

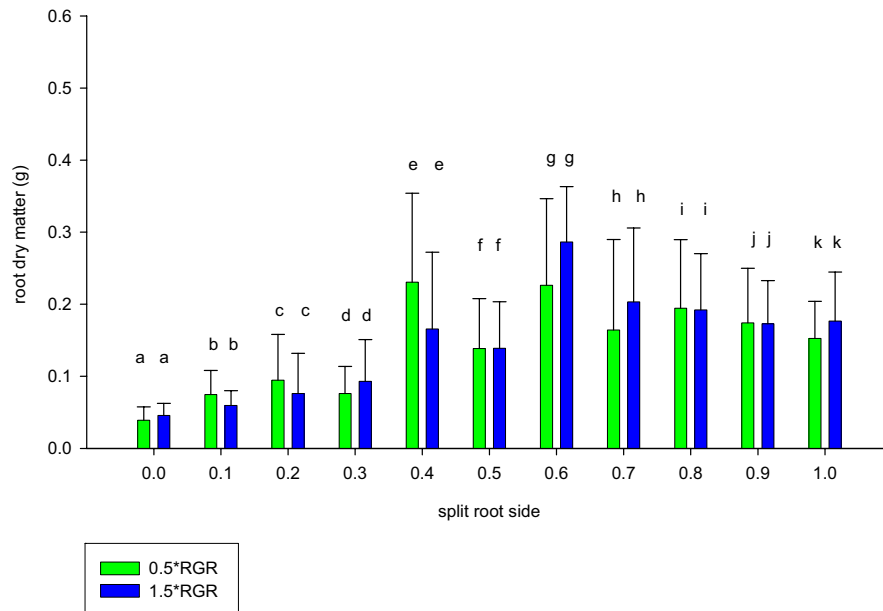


Figure 13: Comparison of the root dry matter of the split root sides at high and low nutrient conditions. Different letters mark statistical significant differences between the respective split root sides of the different experiments. Means and standard deviation are shown in the graphic. Statistical analyses were done by t-test ($n=9$; $p<0.05$; for split root side 0.5 $n=18$; $p<0.005$).

Nutrient heterogeneity had an impact on the root masses within the treatments. If the nutrient addition was adapted to 0.5*RGR the plants first responded with different root masses at the 0.7/0.3 treatment. In the high nutrition experiment the plants responded already at the 0.6/0.4 treatment. However, the comparison of the root dry matter of the split root sides between the high and low nutrition experiment showed no differences. Generally, if plants responded to an unequal supply of nutrients, root sides which got more nutrients had higher root biomass than the respective root sides with fewer nutrients. Furthermore, this response was independent of the concentration of the nutrient solution within the experiment.

3.1.3.5 Shoot/root ratio

Because of the equal shoot and total root biomass, the shoot/root ratios did not differ between the treatments within the experiments (Figure 14).

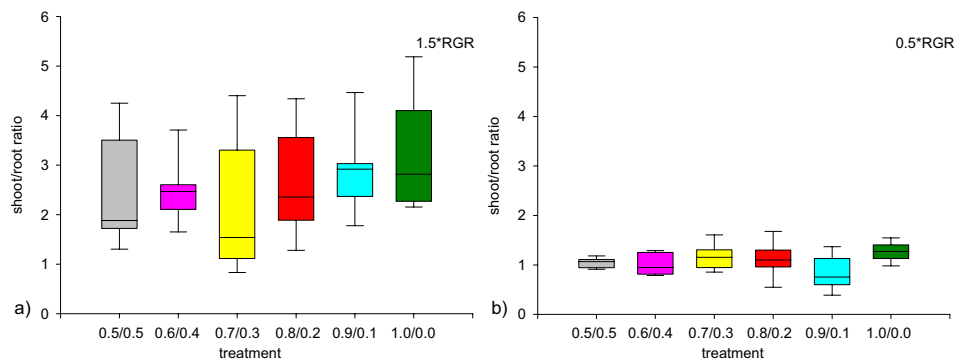


Figure 14: Comparison of the shoot/root ratios at high and low nutrient conditions. Statistical analyses were done by a ONE-WAY-ANOVA ($n=9$; $p<0.05$).

The comparison of the shoot/root ratios showed that in the experiment with a high amount of nutrients ratios were higher than in the other experiment because of the higher shoot and lower root biomass (Figure 10; 11). It was detected that the nutrient status of the plants influenced the shoot/root ratio, but the unequal distribution of nutrients did not.

3.1.4 Root structure

3.1.4.1 Root surfaces

The comparison of the root surfaces in the experiment under high nutrient condition (1.5*RGR) showed, that there were differences between the heterogeneity treatments (Figure 15a). In the control treatment (0.5/0.5) the surfaces on the right root side were higher than on the left. In all other treatments the right split root sides, which got less nutrients, had smaller root surfaces than at the other side, which got a higher concentrated nutrient solution. These differences were not visible in the comparison of the total root surfaces (Figure 15c), where the root surfaces of the two split root sides in the treatments were summed up. Here the treatments did not differ.

The comparison of the root surfaces in the treatments in the experiment with a lack of nutrients (0.5*RGR) showed that the root surfaces of the control treatment (0.5/0.5) were about the same size (Figure 15b). At the following treatments root surfaces of the two split root sides were different. As before in the other experiment, root sides which got a higher concentrated nutrient solution had higher surfaces as the other root sides. Total root surfaces in treatment 0.5/0.5 were nearly two times higher than in the treatment 1.0/0.0 (Figure 15d). Because of the high variability the treatments 0.7/0.3 and 0.8/0.2 were not different from each other as well as from the control (0.5/0.5).

It was striking that the total root surfaces of the control treatments in both experiments were nearly about the same size.

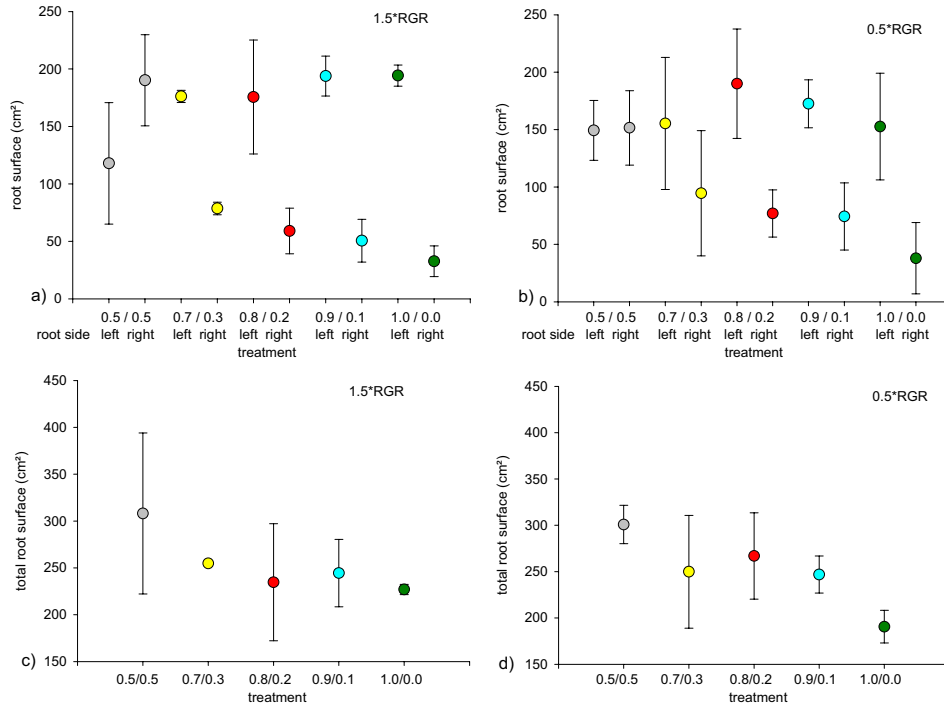


Figure 15: Comparison of the root surfaces in and between the treatments at high and low nutrient conditions. Means and standard deviations are shown in the graphics (n=3).

3.1.4.2 Root length

Within the 1.5*RGR experiment root length (Figure 16a) of the control treatment (0.5/0.5) differed in the same way as root surfaces did. In the other treatments roots were longer at the side which got a higher concentration of nutrients than at the respective root side of the treatment. The total root lengths of the treatments were nearly equal (Figure 16c). On average the total root had a length of 958.8 ± 139.3 cm.

With a limited amount of nutrients (0.5*RGR) the root length in the treatments first differed at the 0.8/0.2 treatment (Figure 16b). The other treatments showed that the root sides which got a higher concentration of nutrients had a higher root length compared to their other root side with a lower nutrient concentration. The total root length was the highest at the control treatment. The other treatments had a lower total root length (Figure 16d). The total root length was on average 957.4 ± 160.8 cm.

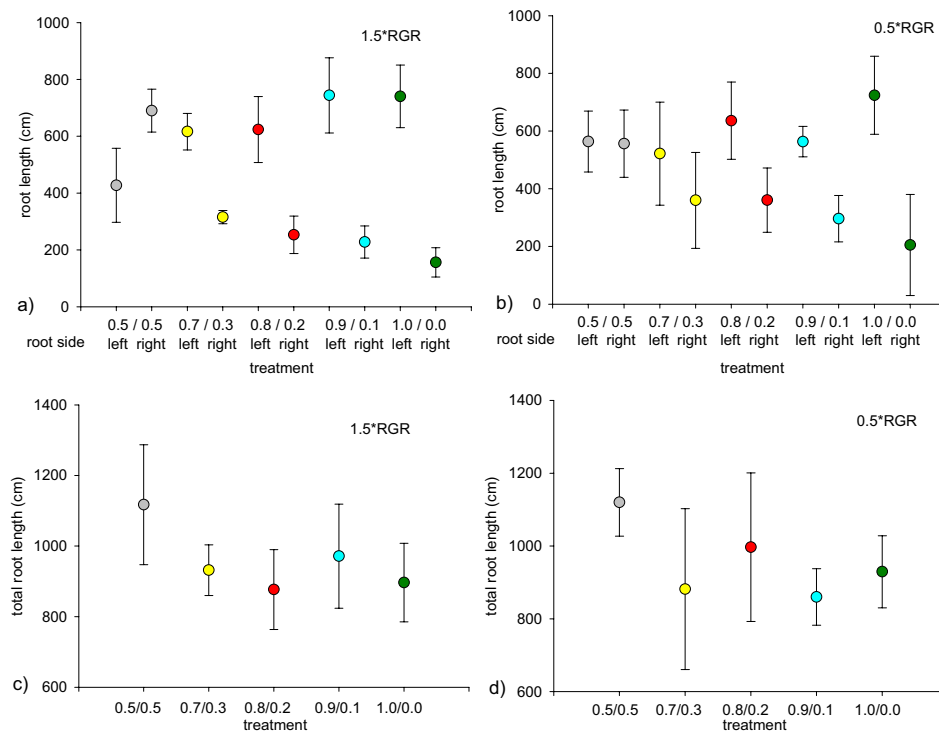


Figure 16: Comparison of the root length in and between the treatments at high and low nutrient conditions. Means and standard deviations are shown in the graphics (n=3).

Nutrient heterogeneity had an impact on the total and single split root surfaces and lengths. The left root sides, which got a higher concentration of nutrient solution, had higher surfaces and lengths as the respective right root sides. The different nutrition levels of the two experiments had little influence on root surfaces and lengths. In the experiment with 1.5*RGR the differences between the two root sides were more distinct than in the 0.5*RGR experiment.

3.1.4.3 Specific root length

Under high nutrient conditions (1.5*RGR) the specific root length (root length/root mass) was uniform in the different treatments (Figure 17a) as well as the total specific root length (Figure 17c) which was on average 45.15 ± 9.53 m/g.

In the 0.5*RGR experiment the specific root length was about the same (Figure 17b) as in the 1.5*RGR experiment. But the specific root length differed within the 0.8/0.2 treatment of the 0.5*RGR experiment. The specific root lengths within the other treatments were mostly equal because of the high variability of the plants.

Only the 1.0/0.0 treatment of the low nutrition experiment (Figure 17d) had a higher specific root length (68.33 ± 11.29 m/g) than the other treatments (46.28 ± 6.21 m/g).

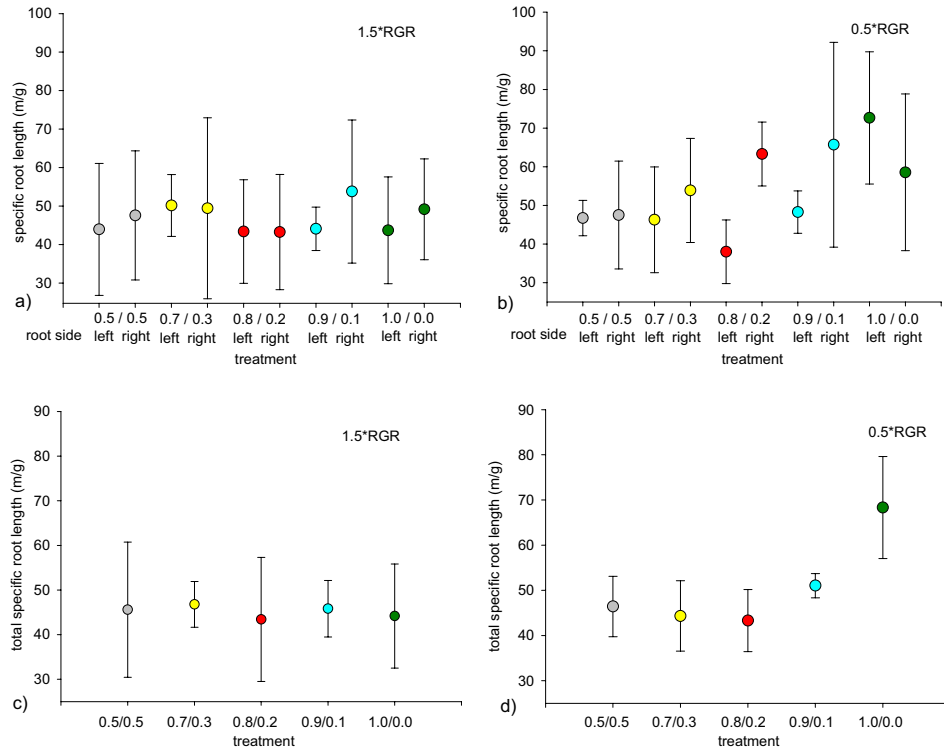


Figure 17: Comparison of the specific root length in and between the treatments at high and low nutrient conditions. Means and standard deviations are shown in the graphics (n=3).

Neither nutrient heterogeneity nor the nutrition status of the plants had a detectable impact on the specific root length.

3.1.5 Findings of the structural parameters

The nutrient status was the major factor in plant development, although the actual states of the leaves decreased in the experiments. Plants, which were adapted to 1.5*RGR developed more and healthier leaves with a higher leaf area than plants with a lack of nutrients. Nutrient heterogeneity had no impact on these factors (table 2).

In both experiments the total biomass were nearly equal. There was no impact of the nutritional level and unequal distribution of nutrients on the total biomass level. But the experiments differed in shoot/root ratio, because shoot masses of the plants with an insufficient nutrient supply were lower. This was caused by a reduced number of leaves, which were smaller compared with leaves of good fertilized plants. The nutrient status had an impact on the shoot/root partitioning in plants. In contrast to this, the unequal distribution of nutrients had no effect.

The response to nutrient heterogeneity at the root side biomass level depended on the nutrition status of the plants. Plants with a high addition rate of nutrients responded more sensitive (first inequality in root biomass at treatment 0.6/0.4) than plants with a lack of nutrients (response at treatment 0.7/0.3).

The unequal distribution of nutrients influenced both - root surface and root length within the treatments. Root sides, which got a higher concentration of nutrients, had a higher root surface and length than the respective other root sides. Different nutrition levels of the plants had only little effect on root structure. Differences between the two root sides were clearer in the experiment with a high nutrient level than in the experiment with 0.5*RGR.

The specific root length was not influenced by nutrient heterogeneity and nutrition status because the plants showed for the parameters root lengths and biomass high variability.

In table 2 all structural parameters were summarized. Just 25 % of the parameters in the experiments showed an impact of nutrient heterogeneity. But in 41.6 % of all structural parameters an effect of the plant nutrient status could be detected.

Table 2: Summary of the impact of nutrient heterogeneity and nutrient level on maize plants.

1 = impact, 0 = no impact. Total number of structural parameters = 12.

		Impact of nutrient heterogeneity		Impact of nutrient level
		1.5*RGR	0.5*RGR	
Structural	Development of the plants	0	0	1
	Average actual states of the leaves	0	0	1
	Leaf area	0	0	1
	Shoot biomass	0	0	1
	Total root biomass	0	0	0
	Root biomass left & right	1	1	1
	Root surface left & right	1	1	0
	Total root surface	0	0	0
	Root lenght left & right	1	1	0
	Total root length	0	0	0
	Spefic root length left & right	0	0	0
	Total specific root length	0	0	0
% of total structural parameters		25	25	41.6

3.2 Functional Parameters

3.2.1 Development of the plants in the experiments: functional measurements

3.2.1.1 Photosynthesis

During the experiments the rate of photosynthesis was measured in both experiments. In the high nutrition experiment (Figure 18a) the treatments had an unique rate of photosynthesis. The rates of photosynthesis were nearly the same at the various measurement days. But at the end of the experiment the rate of photosynthesis slightly decreased in all experiments.

In the experiment with 0.5*RGR only once (day 8) a statistical significant difference of the rate of photosynthesis between the treatments was found (Figure 18b). Afterwards the rate of photosynthesis did not differ. During the experimental period the photosynthesis rate decreased.

The comparison of the photosynthesis rate between plants with different nutrient levels indicated that at the beginning of the experiment the rate of photosynthesis was nearly equal. But it decreased faster in plants with a lack of nutrients than in well fertilized plants. This trend continued until the end of the experiment. Finally plants adapted to 0.5*RGR had a lower photosynthesis rate than plants with a nutrient level of 1.5*RGR.

In both experiments the rate of photosynthesis was not affected by nutrient heterogeneity.

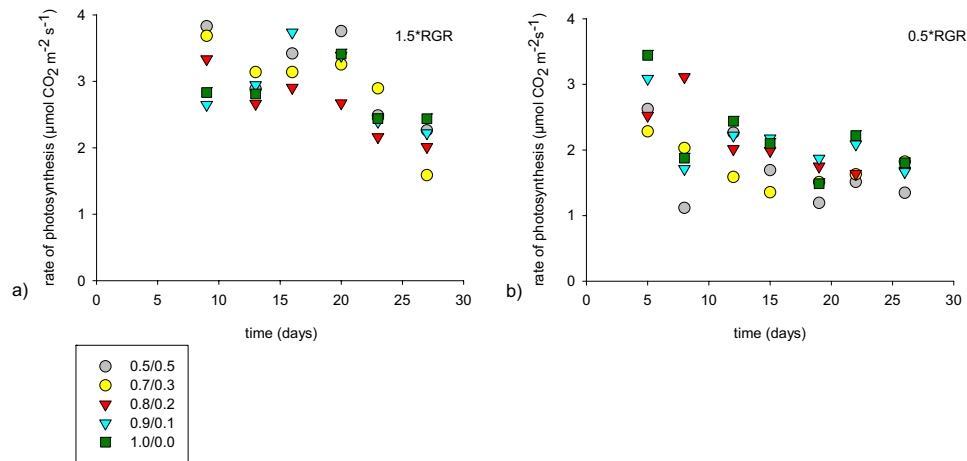


Figure 18: The rate of photosynthetic CO₂ uptake of plants at high and low nutrient conditions. Means are shown in the graphic without standard deviations, because the treatments did not statistically differ. Statistical analyses were done by ONE-WAY-ANOVA (n=5; p<0.05) or if normality test failed by Kruskal-Wallis One Way Analyses of Variances on Ranks (n=5; p<0.05).

3.2.1.2 Chlorophyll

After the harvest of plants chlorophyll concentrations of the leaves were measured. The chlorophyll concentration in the experiment with 1.5*RGR did not differ significantly between the treatments because of the high standard deviation (Figure 19a). On average the chlorophyll concentration was 1.58 ± 0.8 (mg/g).

In the experiment with 0.5*RGR the standard deviation was lower but the chlorophyll concentration did not differ (Figure 19b). The chlorophyll concentration was on average 0.65 ± 0.14 (mg/g).

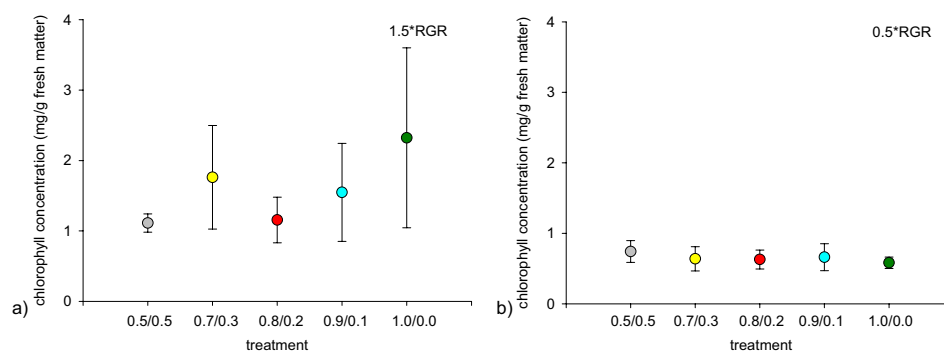


Figure 19: Chlorophyll concentration of plants at high and low nutrient conditions. Means and standard deviations are shown in the graphics. Statistical analyses were done by ONE-WAY-ANOVA (n=4; $p < 0.05$).

The nutrition level of plants had an impact on the chlorophyll concentration of the leaves: plants with a high nutrient supply had a higher concentration than plants at low nutrient conditions. However, it seemed that nutrient heterogeneity had no impact on chlorophyll concentration.

3.2.2 Internal nutrient concentration

From 2 plants of both experiments further internal nutrients were measured. It resulted that the dry matter of the plants contained 40 % O₂, 55 % H, 10 % diverse macro and micro nutrients (Ca, Fe, K, Na, Mg, Mn, Si, Cl). The sum of carbon, nitrogen and sulphur was nearly 44 %. Therefore C, N and S concentrations were measured more in detail.

3.2.2.1 C/N ratio

With increasing nutrient heterogeneity the C/N ratios of the total plants decreased (Figure 20e, f). Independent of the nutrient level the 1.0/0.0 treatments were statistically significant different from the other treatments. The ranges of the C/N ratios in both experiments were different. In the 1.5*RGR experiment the C/N ratio of the total plant ranged from 12.9 to 17.6. In contrast to this in the 0.5*RGR experiment it ranged from 32.1 to 40.9.

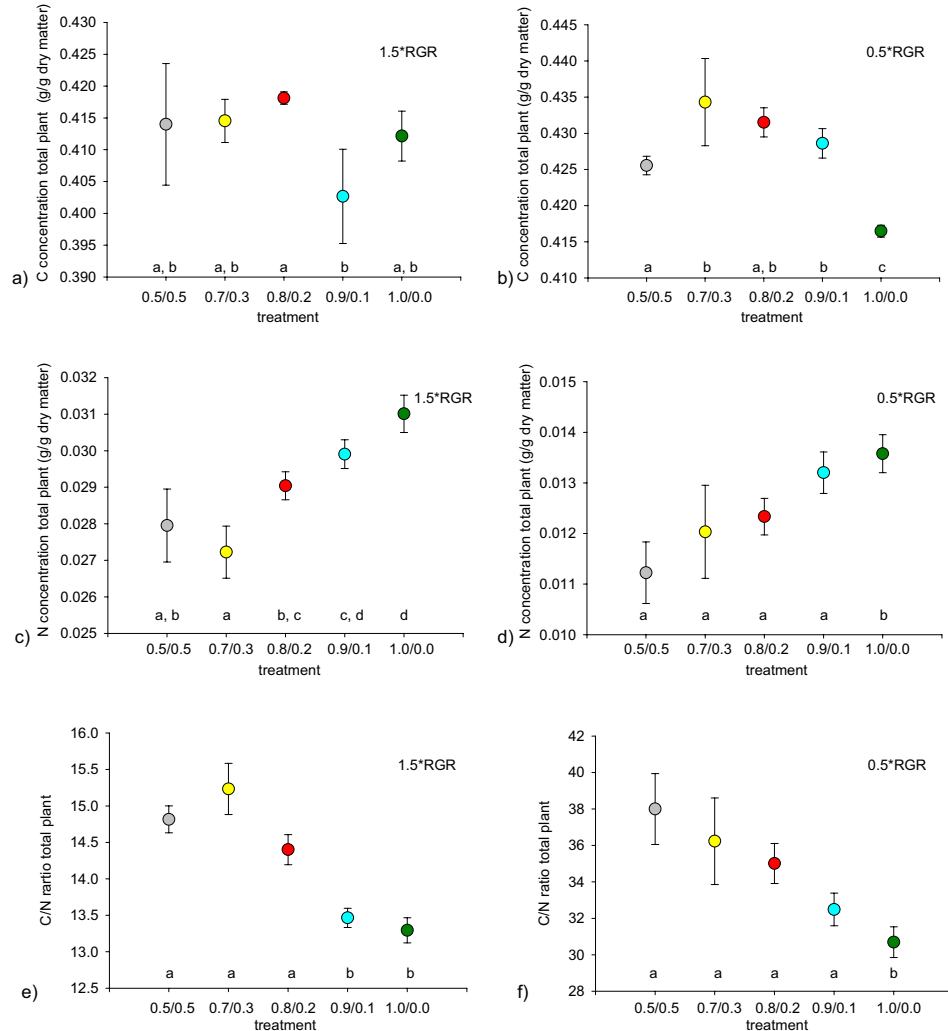


Figure 20: C concentrations (a, b), N concentrations (c, d) and C/N ratios (e, f) of the total plants at high and low nutrient conditions (1.5*RGR, 0.5*RGR). Means and standard deviations are shown in the graphics. Different letters mark statistical significant differences between the treatments. Y-axes are not equal. Statistical analyses were done by One Way ANOVAs ($n=4$, $p<0.05$) or if normality test failed by Kruskal-Wallis One Way Analyses of Variances on Ranks ($n=4$; $p<0.05$). As the pair wise multiple comparison procedure a Tukey test was used.

Nutrient heterogeneity had an impact on the total plant C/N ratios (Figure 20e, f). The C/N ratios were the lowest in the treatments with the highest inequality of nutrient supply (1.0/0.0 treatments). Furthermore, the different nutrition levels had an impact on the C/N ratios. Plants which got a high amount of nutrients had a closer C/N ratio than plants with an insufficient nutrient supply.

The C concentrations in the shoots were nearly constant (Figure 21a, b) for both nutrition experiments. But the 0.9/0.1 treatment of the 1.5*RGR experiment had lower C concentrations compared to all other treatments. In the 0.5*RGR experiment the 1.0/0.0 treatment had lower C concentration than the remaining treatments. However, in the experiments the C concentrations were about the same size.

If the N concentrations of the shoots were compared between the treatments (Figure 21c, d), it could be detected that the N concentration was increasing with inequality of nutrient supply. In the 1.5*RGR as well as in the 0.5*RGR experiment the 1.0/0.0 treatment had the highest N concentration and differed statistically significant from most of the other treatments.

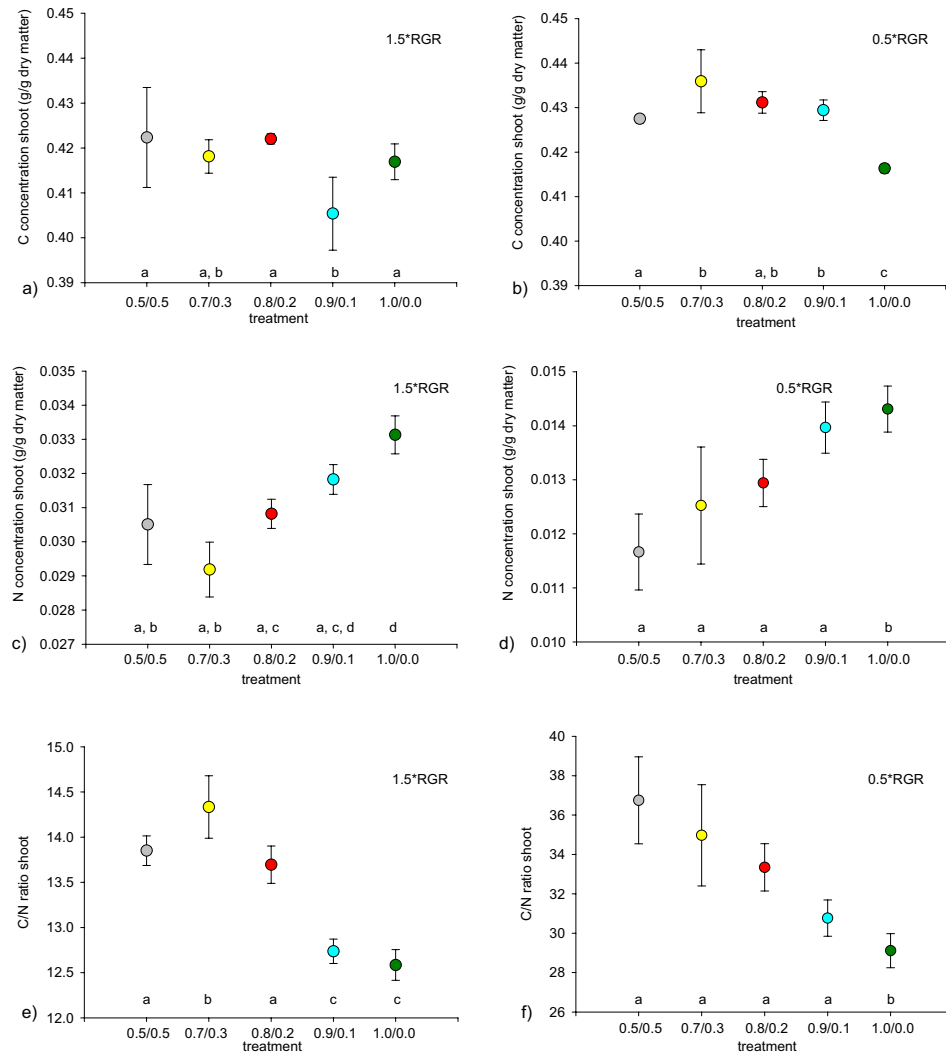


Figure 21: C concentrations (a, b), N concentrations (c, d) and C/N ratios (e, f) of the shoots of plants at high and low nutrient conditions (1.5*RGR, 0.5*RGR). Means and standard deviations are shown in the graphics. Different letters mark statistical significant differences between the treatments. Y-axes are not equal c) - f). Statistical analyses were done by One Way ANOVAs ($n=4$, $p<0.05$) or if normality test failed by Kruskal-Wallis One Way Analyses of Variances on Ranks ($n=4$; $p<0.05$). As the pair wise multiple comparison procedure a Tukey test was used.

As a consequence of different nutrition conditions plants showed different levels of N concentrations in the shoots. Rising nutrient heterogeneity correlated with increasing N concentrations and decreasing C/N ratios in shoots (Figure 21e, f). This response to nutrient heterogeneity was independent of nutrient status.

C concentrations of the total root in the 1.5*RGR experiment were unequal (Figure 22a). The control treatment had the lowest C concentration. The opposite of the control was the 0.7/0.3 treatment. Here the highest C concentration was detected. From the 0.7/0.3 treatment the C concentration decreased with the increase of inequality of nutrient supply. Furthermore, the N concentration of the total root was non-uniformly distributed in the 1.5*RGR experiment, too (Figure 22c). The lowest N concentration could be detected in the control treatment and differed from all other treatments. The 0.9/0.1 and 1.0/0.0 treatments were not statistically significant different.

As a consequence of the variable C and N concentrations of the total root in the 1.5*RGR experiment, the C/N ratio was non-uniformly distributed, too (Figure 22e). C/N ratios of the total root could be divided into two different groups. One group was formed by the 0.5/0.5 and 0.7/0.3 treatment. Here, the distribution of nutrients caused no differences in the C/N ratios. The C/N ratios were higher than in the second group. This group was formed by all other treatments. Here, nutrient heterogeneity caused lower C/N ratios and significant differences between the treatments within this group.

In the 0.5*RGR experiment, the C concentrations of the total roots increased from the control to the 0.8/0.2 treatment. Here the C concentration was the highest. Then the C concentrations fell to a level of the 0.5/0.5 treatment. Because of the statistics the C concentrations of the total root could be regarded as uniformly (Figure 22b).

Such a pattern as for the C concentrations was not shown for the N concentrations of the total root (Figure 22d). Here the treatments could be divided in two different groups. One group had a higher (0.5/0.5, 0.7/0.3, 0.8/0.2) and one group a lower N concentration (0.9/0.1, 1.0/0.0).

Because of the different N concentrations, the C/N ratios of the total root had to be formed in two different groups, too (Figure 22f). The first group was created by the treatments 0.5/0.5, 0.7/0.3 and 0.8/0.2. These treatments had lower C/N ratios. On the other hand the C/N ratios were higher in 0.9/0.1 and 1.0/0.0 treatments which formed the other group.

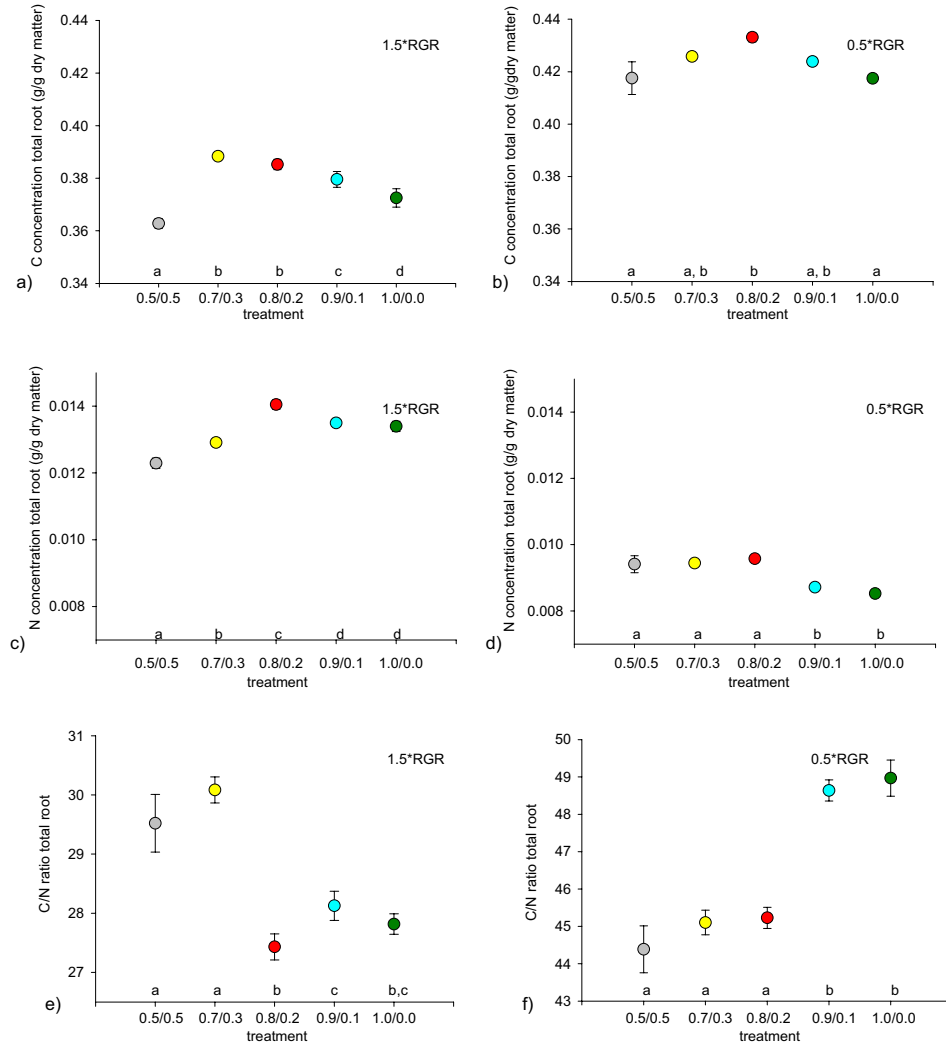


Figure 22: C concentrations (a, b), N concentrations (c, d) and C/N ratios (e, f) of the total roots at high and low nutrient conditions (1.5*RGR, 0.5*RGR). Means and standard deviations are shown in the graphics. Different letters mark statistical significant differences between the treatments. Y axes are not equal e) - f). Statistical analyses were done by One Way ANOVAs (n=4, p<0.05) or if normality test failed by Kruskal-Wallis One Way Analyses of Variances on Ranks (n=4; p<0.05). As the pair wise multiple comparison procedure a Tukey test was used.

The different nutrient status of the plants had an impact on the C concentration of the total root. A lack of nutrients caused higher C concentrations than a high nutrient supply. The N concentrations were on different levels in the experiments. Plants in the low nutrition experiment had lower N concentrations in their root systems than plants in the high nutrition

experiment. The different nutrition levels of the plants caused different patterns of the N concentration. This was obviously shown in the C/N ratios (Figure 22e, f). Although the plants responded to nutrient heterogeneity with a changing C/N ratio of the total roots in the experiments, plants with a high nutrition had a smaller C/N ratio, if nutrient heterogeneity was high enough. But if plants had a lack of nutrients, the C/N ratios of the roots were higher when nutrient heterogeneity was big.

The C concentrations of the roots in the 1.5*RGR experiment were non-uniform distributed and no trend was obvious (Figure 23a).

The N concentrations of the 1.5*RGR experiment were similar to the control treatment. At the root sides, where higher concentrated nutrient solution was supplied, higher N concentrations were detectable as at the right root sides (Figure 23c).

C and N concentration in the roots ranged from 0.35 to 0.42 (C g/g dry matter) and from 0.01 to 0.015 (N g/g dry matter) respectively. The comparison of the C/N ratios of roots within the treatments (Figure 23e) showed no differences in the control treatment. However, in all other treatments the root sides differed. Only in the 0.7/0.3 treatment the C/N ratio of the left root side was higher than the right. The remaining treatments showed higher C/N ratios at the right root sides.

The C concentrations in the 0.5*RGR experiment were non-uniform and no trend was obvious (Figure 23b). The N concentrations stayed equal for the two root sides in the control treatment (Figure 23d). In all other treatments the left sides which got higher amounts of nutrients had higher N concentrations compared to the respective root side of the treatment. The C/N ratios in that experiment (Figure 23f) showed higher ratios at the right root sides. This was caused by the non-uniform C concentrations (e.g. 0.5/0.5) and the fact that the N concentrations were lower at the right root sides (e.g. 0.8/0.2).

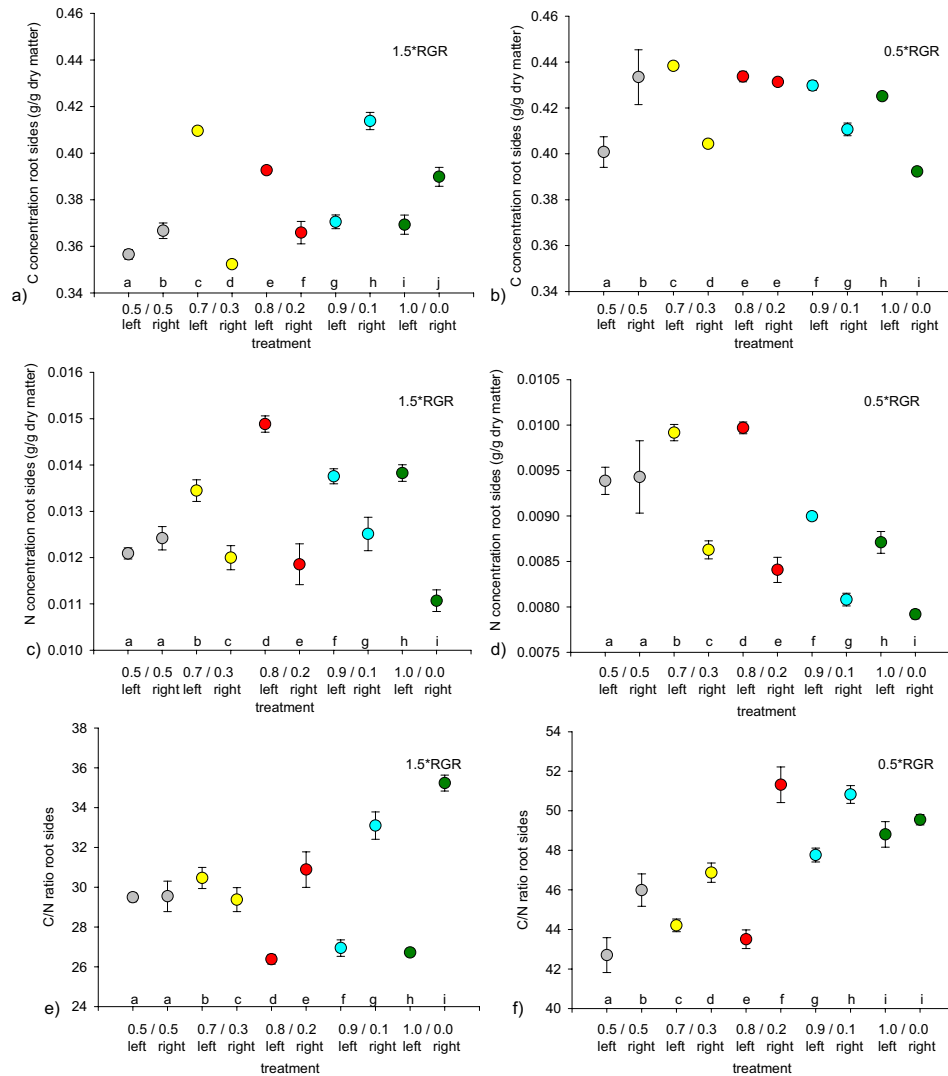


Figure 23: C concentrations (a, b), N concentrations (c, d) and C/N ratios (e, f) of the left and the right root sides of plants at high and low nutrient level (1.5*RGR, 0.5*RGR). Means and standard deviations are shown in the graphics. Y-axes are not equal c) – f). Different letters mark statistical significant differences within the treatments. Statistical analyses were done by t-tests ($n=4$, $p<0.05$).

In both experiments the C concentrations were non-uniform in all treatments. The different nutrient levels caused nearly equal C concentrations. No impact of nutrient heterogeneity could be detected.

Independent of the nutrient status of the plants, N concentrations of the root sides in the control treatments were similar between the treatments. Higher N concentrations were

determined at the root sides where more nutrients were supplied. Here, the impact of nutrient heterogeneity was detectable. The different nutrition levels in the experiments caused different levels of N concentrations: plants with a lack of nutrients had on average 0.0089 ± 0.0007 N (g/g dry matter) while plants which got a lot of nutrients had 0.013 ± 0.0011 N (g/g dry matter). The N concentration levels in the experiments caused different C/N ratio levels.

3.2.2.2 C/S ratio

The S concentrations and the C/S ratios of the total plant were uniform but on different levels. Plants with a high nutrient supply had higher S concentrations and smaller C/S ratios than plants with a lack of nutrients (Figure 24).

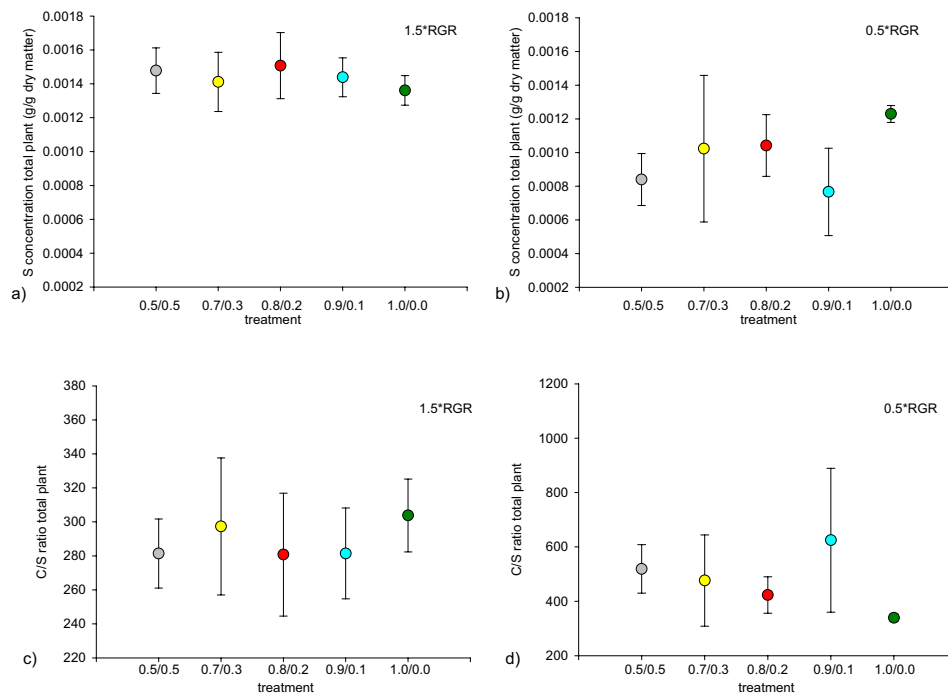


Figure 24: S concentrations (a, b) and C/S ratios (c, d) of the total plants at high and low nutrient conditions (1.5*RGR, 0.5*RGR). Means and standard deviations are shown in the graphics. Different letters mark statistical significant differences between the treatments. Y-axes are not equal (c, d). Statistical analyses were done by One Way ANOVAs ($n=4$, $p<0.05$) or if normality test failed by Kruskal-Wallis One Way Analyses of Variances on Ranks ($n=4$; $p<0.05$). As the pair wise multiple comparison procedure a Tukey test was used.

It had to be recognized that nutrient heterogeneity did not influence the C/S ratios of the roots, shoots and total plants, but the nutrient status of the plants had an impact.

Plants in the 1.5*RGR experiment had on average a higher S concentration (0.0014 ± 0.0002 g/g dry matter) (Figure 25a) in the shoots than plants with a lack of nutrients (0.0009 ± 0.0002 g/g dry matter) (Figure 25b).

Equal C (Figure 21a, b; p. 39) and S concentrations in the shoots caused uniform C/S ratios (Figure 25c, d). In the 1.5*RGR experiment the average C/S ratio was about 297.8 ± 34.3 g/g dry matter in contrast to 544.9 ± 341.2 g/g dry matter in the low nutrition experiment.

Nutrient heterogeneity had no impact on the S concentrations of the shoots but the nutritional level of the experiments affected it.

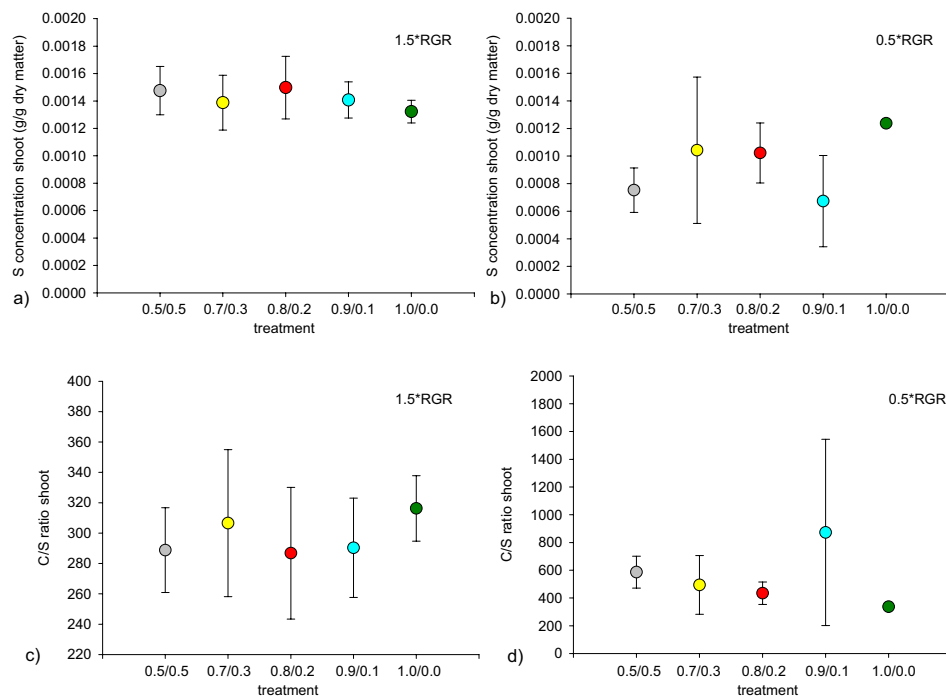


Figure 25: S concentrations (a, b) and C/S ratios (c, d) of the shoots of plants at high and low nutrient conditions (1.5*RGR, 0.5*RGR). Means and standard deviations are shown in the graphics. Different letters mark statistical significant differences between the treatments. Y-axes are not equal (c, d). Statistical analyses were done by One Way ANOVAs ($n=4$, $p<0.05$) or if normality test failed by Kruskal-Wallis One Way Analyses of Variances on Ranks ($n=4$; $p<0.05$). As the pair wise multiple comparison procedure a Tukey test was used.

The S concentrations of the total roots were similar between the experiments (Figure 26a, b). But the different nutrient levels of the experiments caused different average S concentrations. Plants with a high nutrient supply had higher S concentrations (0.0016 ± 0.0002 g/g dry matter) than plants which were adapted to 0.5*RGR (0.0011 ± 0.0002 g/g dry matter). The constant S concentrations as well as the impact of the nutrient status of the plants were reflected in the C/S ratios of the total roots (Figure 26c, d), which were uniform.

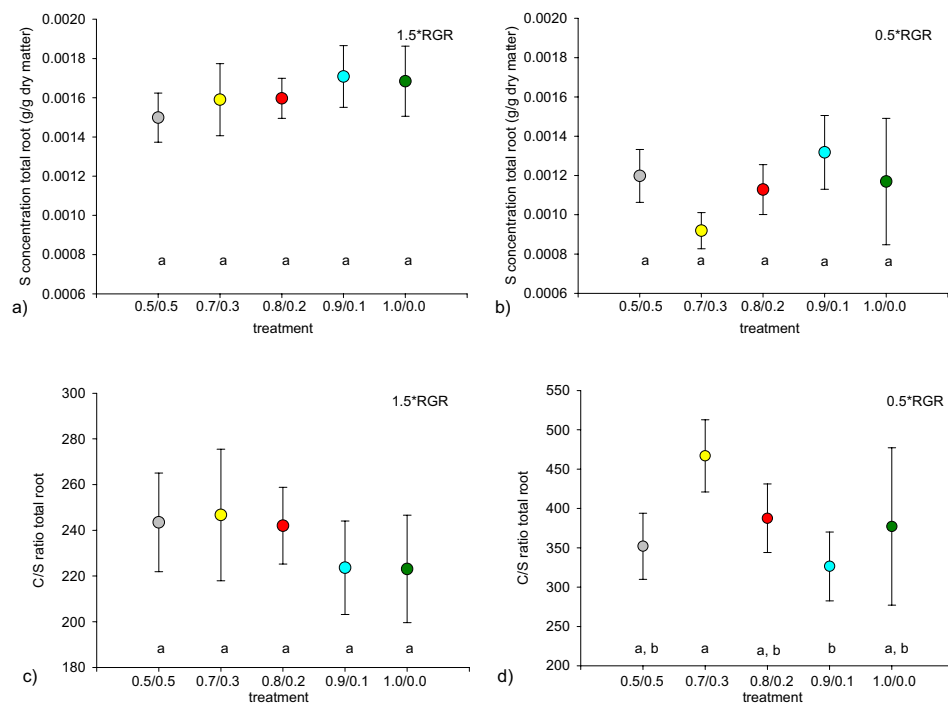


Figure 26: S concentrations (a, b) and C/S ratios (c, d) of the total roots of plants at high and low nutrient conditions (1.5*RGR, 0.5*RGR). Means and standard deviations are shown in the graphics. Different letters mark statistical significant differences between the treatments. Y-axes are not equal (c, d). Statistical analyses were done by One Way ANOVAs ($n=4$, $p<0.05$) or if normality test failed by Kruskal-Wallis One Way Analyses of Variances on Ranks ($n=4$; $p<0.05$). As the pair wise multiple comparison procedure a Tukey test was used.

The S concentrations at the root sides in the control treatments were different in the 1.5*RGR as well as in the 0.5*RGR experiment (Figure 27a, b). Generally, the S concentrations of the roots varied in the experiments. The concentrations were on average 0.0016 ± 0.0003 (g/g dry matter) in the 1.5*RGR and 0.0011 ± 0.0003 (g/g dry matter) in the 0.5*RGR experiment.

The C concentrations for the root sides were described in (Figure 23 a, b; p. 43). Because of the non-uniform C and S concentration at the root sides the C/S ratios were different

(Figure 27c, d). The C/S ratios of the 1.5*RGR experiment were smaller than those of the 0.5*RGR experiment.

The nutrition status of the plants affected S concentration levels in the roots, but nutrient heterogeneity did not.

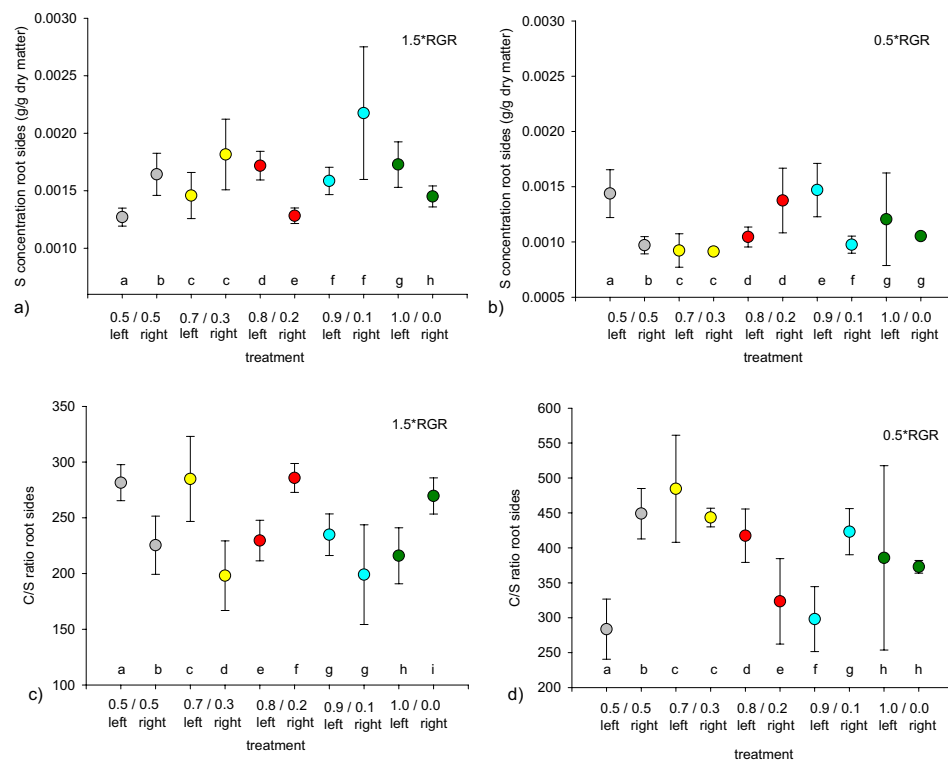


Figure 27: S concentrations (a, b) and C/S ratios (c, d) of the different split root sides of plants at high and low nutrient conditions (1.5*RGR, 0.5*RGR). Means and standard deviations are shown in the graphics. Different letters mark statistical significant differences between the treatments. Y-axes are not equal (c, d). Statistical analyses were done by One Way ANOVAs ($n=4$, $p<0.05$) or if normality test failed by Kruskal-Wallis One Way Analyses of Variances on Ranks ($n=4$; $p<0.05$). As the pair wise multiple comparison procedure a Tukey test was used.

3.2.3 Metabolites

3.2.3.1 Sugars and Starch

Within each experiment the concentration of glucose, fructose, sucrose and starch in leaves and roots was not statistical significant different between the treatments (Figure 28a, b; 29a, b).

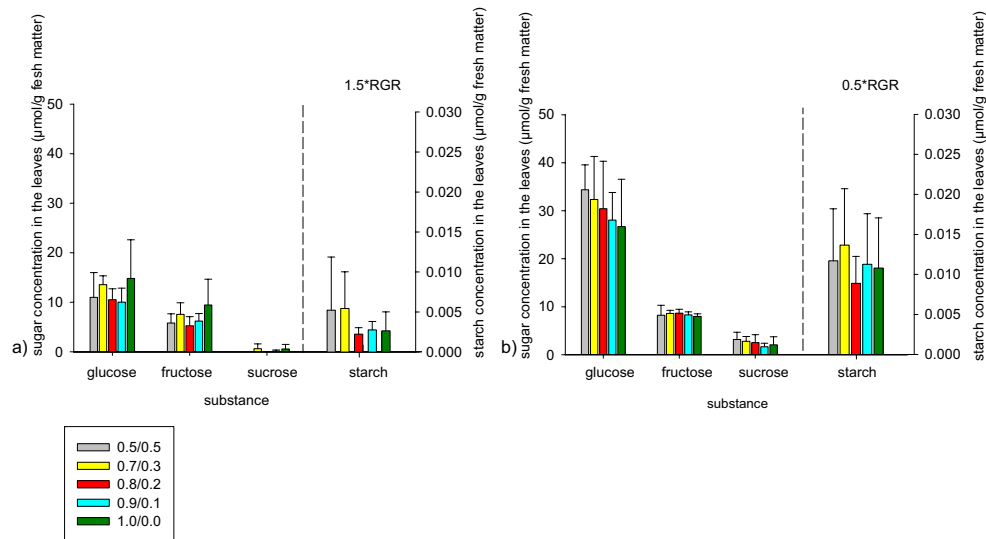


Figure 28: The concentration of glucose, fructose, sucrose and starch in the leaves of plants at high and low nutrition conditions. Means and standard deviations are shown in the graphics. There were no statistically significant differences between the treatments for the respective substance. Statistical analysis were done by ONE-WAY-ANOVA ($n=4$; $p<0.05$) or if normality test failed by Kruskal-Wallis One Way Analyses of Variances on Ranks ($n=4$; $p<0.05$).

In the low nutrition experiment the concentrations of glucose in the leaves (Figure 28b) ($30.38 \pm 7.82 \mu\text{mol/g fresh matter}$) were much higher than in the high nutrition experiment ($11.97 \pm 4.44 \mu\text{mol/g fresh matter}$) (Figure 28a). In both experiments fructose concentrations were nearly the same. Sucrose concentrations in the high and low nutrition experiment were different between the experiments. In the 1.5*RGR experiment the leaf sucrose concentration was on average $0.25 \pm 0.61 (\mu\text{mol/g fresh matter})$. The sucrose concentration in the 0.5*RGR experiment was much higher ($2.44 \pm 0.61 \mu\text{mol/g fresh matter}$). The concentrations of starch in the leaves were low in both experiments compared to the glucose concentrations.

Nutrient heterogeneity had no impact on the concentration of glucose, fructose, sucrose and starch in the leaves.

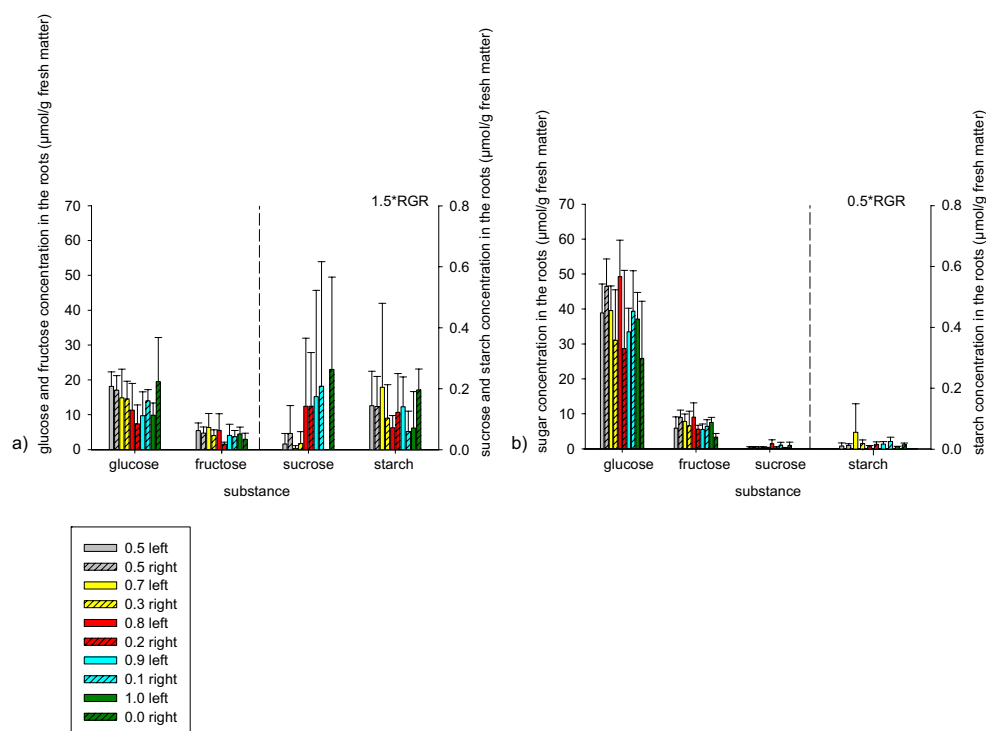


Figure 29: The concentration of glucose, fructose, sucrose and starch in the roots of plants at high and low nutrient conditions. Means and standard deviations are shown in the graphics. There were no statistically significant differences between the treatments for the respective substance. Statistical analyses were done by ONE-WAY-ANOVA ($n=4$; $p<0.05$) or if normality test failed by Kruskal-Wallis One Way Analyses of Variances on Ranks ($n=4$; $p<0.05$).

The glucose concentrations of the roots (Figure 29) in the low nutrition experiment were higher ($36.95 \pm 12.90 \mu\text{mol/g}$ fresh matter) than in the high nutrition experiment ($13.63 \pm 7.00 \mu\text{mol/g}$ fresh matter). In both experiments the fructose concentrations of the roots were nearly the same. The sucrose concentrations of the roots were higher in the experiments with a lack of nutrients ($0.5 \times \text{RGR}$) ($0.54 \pm 0.65 \mu\text{mol/g}$ fresh matter) but not the starch concentrations ($0.02 \pm 0.03 \mu\text{mol/g}$ fresh matter). In the high nutrition experiment the sucrose concentrations of the roots were on average $0.10 \pm 0.21 \mu\text{mol/g}$ fresh matter) whereas the starch concentrations were about $0.12 \pm 0.12 \mu\text{mol/g}$ fresh matter).

Nutrient heterogeneity had no impact on the concentration of glucose, fructose, sucrose and starch in the roots.

The TNC concentration (Total Non-structural Carbohydrate; sum of glucose, fructose, sucrose and starch) in the leaves and in the roots did not statistically significant differ (Figure 30a - d).

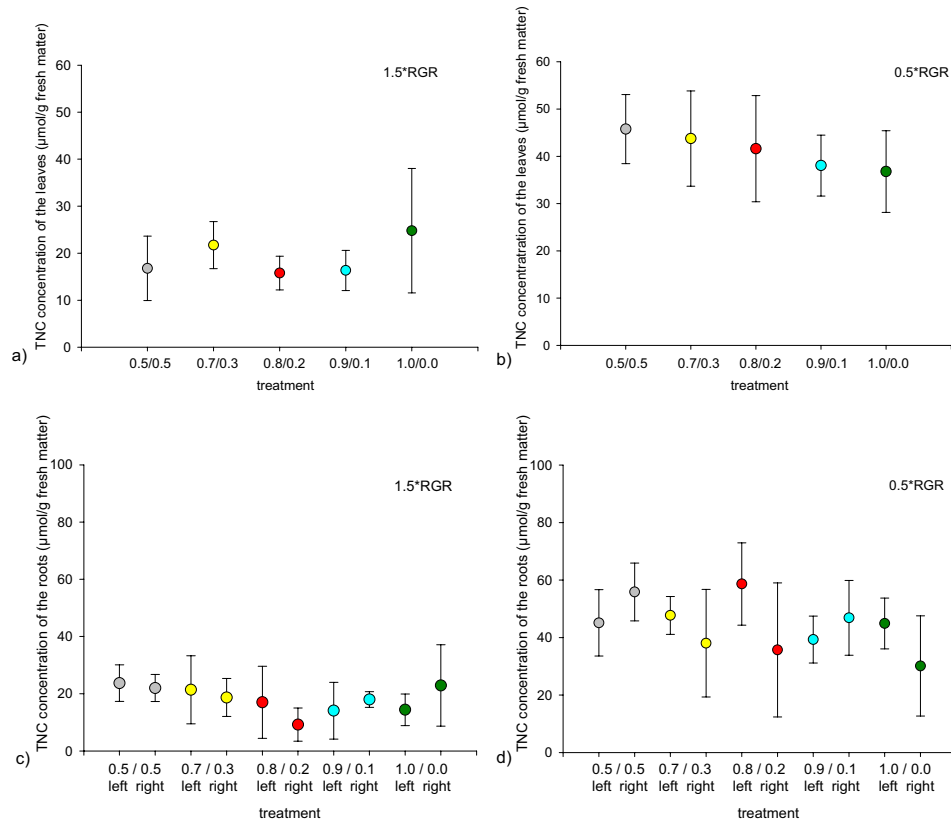


Figure 30: Total Non-structural Carbohydrate (TNC) concentration in the leaves and roots of plants at high and low nutrient conditions. Means and standard deviation are shown in the graphics. There were no statistically significant differences between the treatments. Statistical analyses were done with by ONE-WAY-ANOVA ($n=4$; $p<0.05$) or if normality test failed by Kruskal-Wallis One Way Analyses of Variances on Ranks ($n=4$; $p<0.05$).

In the 1.5*RGR experiment the TNC concentrations in leaves and in roots were much lower than in the 0.5*RGR experiment. These differences were mainly caused by the different glucose concentrations. Also the experiments differed in the concentrations of fructose, sucrose and starch (Figure 31a, b).

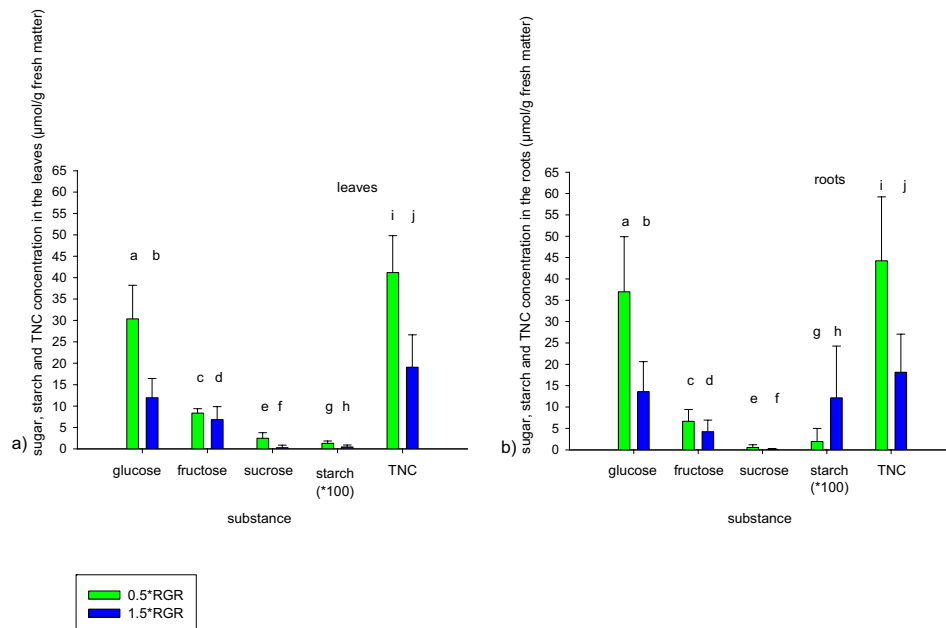


Figure 31: Sugar, starch and TNC concentration of leaves (a) and roots (b) of plants at high and low nutrient conditions. Means and standard deviations are shown in the graphics. Different letters within a substance mark statistical significant differences. Data of the treatments in the respective experiments did not statistically significant differ and were pooled. Because of the small starch concentrations it was necessary to multiply them with factor 100 in order to present them in the graphic. Statistical analyses were done by t-test ($n_{\text{leave}}=20$, $n_{\text{root}}=40$, $p<0.05$) or if normality test failed by Mann-Whitney Rank Sum Test ($n_{\text{leave}}=20$, $n_{\text{root}}=40$, $p<0.05$).

Plants with a lack of nutrients (0.5*RGR experiment) in most cases had higher sugar, starch and TNC concentrations in the roots as well as in the leaves than well fertilized plants (Figure 31). Only once the starch concentration in the roots (Figure 31b) of the plants adapted to 1.5*RGR, was higher than in plants which were adapted to 0.5*RGR. Overall the starch concentrations were much lower than the glucose, fructose and sucrose concentrations.

For the concentration of sugar and starch in leaves and roots nutrient heterogeneity had no significant impact in contrast to the nutrition level of the plants. Plants which were adapted to a low nutrient level had higher sugar and starch concentrations in leaves and roots than plants which were supplied with higher amounts of nutrients.

3.2.3.2 Free amino acids

In the high nutrition experiment the concentrations of alanine (ALA), aspartate (ASP) and glutamate (GLU) were the highest of all described amino acids (Table 3). Only 7 of 18 amino acids were influenced by nutrient heterogeneity.

In the low nutrition experiment the same amino acids as in the 1.5*RGR experiment (alanine, aspartate, glutamate) were the highest of all measured amino acids (Table 3). But in the 0.5*RGR experiment more amino acids were influenced by nutrient heterogeneity (11 out of 18). In the experiment where the nutrient supply was adapted to 0.5*RGR the concentrations were much lower compared with the high nutrition experiment.

Table 3: Free amino acid concentrations in the leaves of plants under high and low nutrient conditions. Means and standard deviations are shown. Statistical significant differences within an experiment are red. Statistical analyses were done by t-test between the treatments (n = 16 respectively n = 12 in the control treatment of the 0.5*RGR experiment; p<0.05).

Amino acid concentration in (µg/g)	1.5*RGR				0.5*RGR			
	0.5/0.5		1.0/0.0		0.5/0.5		1.0/0.0	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
ALA	270.9	29.7	259.8	80.7	20.7	4.0	36.3	11.2
GLY	10.4	1.9	11.3	4.6	1.9	0.4	1.9	0.3
VAL	5.4	0.6	5.2	1.0	2.6	0.1	3.1	0.5
LEU	3.4	0.7	4.0	1.0	1.5	0.1	1.8	0.4
ILEU	2.7	0.3	3.1	0.7	1.1	0.0	1.2	0.2
THR	9.6	1.9	13.4	4.4	1.2	0.1	1.9	0.5
SER	16.9	4.3	27.6	8.7	1.9	0.8	1.9	0.6
PRO	4.5	1.2	4.8	0.8	1.4	0.2	1.8	0.2
ASN	2.5	0.2	2.8	0.7	0.5	0.0	0.7	0.0
ASP	59.1	16.2	112.8	31.3	10.1	1.8	15.3	3.4
MET	0.8	0.2	1.0	0.1	0.4	0.1	0.5	0.1
GLU	194.2	19.7	255.3	37.8	50.5	7.2	53.4	5.2
PHE	3.1	0.6	3.3	0.6	2.1	0.3	2.1	0.4
GLN	19.8	7.1	15.9	1.4	2.9	1.2	3.9	1.1
LYS	3.6	0.7	3.7	0.9	1.1	0.1	1.2	0.3
HIS	1.1	0.5	1.6	1.5	0.4	0.3	0.4	0.1
TYR	2.6	0.7	2.8	1.1	1.5	0.4	1.7	0.2
TRP	0.6	0.2	0.5	0.1	0.4	0.1	0.3	0.0
Total	611.3	33.9	729.0	131.3	102.5	9.7	129.6	12.7

In the high as well as in the low nutrition experiment the total concentrations of the amino acids were different between the control and 1.0/0.0 treatments (Table 3). The nutrition status of the plants influenced the total concentrations of free amino acids in the leaves. Well fertilized plants had higher total concentrations of free amino acids than plants with a lack of nutrients.

Nutrient heterogeneity and the nutrition status of plants had an impact on plants at the level of free amino acids in the leaves.

3.2.4 Findings of the functional parameters

Photosynthesis, chlorophyll concentration of the leaves as well as the products of the primary metabolism were not influenced by nutrient heterogeneity. However, the nutrient status of the plants caused different results. Plants grown under low nutrient conditions showed a lower rate of photosynthesis and chlorophyll concentration than plants in the high nutritional experiment. Also the sugar and starch concentrations in leaves and roots in plants which were fertilized with a low amount of nutrients differed to the plants in the 1.5*RGR experiment. It was striking that the glucose concentration in plants with a lack of nutrients was much higher than in the well fertilized plants.

The results of the plant internal nutrient concentrations showed, that nutrient heterogeneity affected the N but not the C and S concentrations. Generally, N concentrations were higher at root sides which had a higher nutrient supply. This was independent of the nutrient status of the plants. The nutrient status caused the different levels of N concentrations. Plants had a high internal N concentration, if the given total amount of N was high.

Furthermore, N concentration of the shoots was increasing with the inequality of nutrient supply. Similar to the shoot, the N concentration of the total plant rose with increasing nutrient heterogeneity. It is possible that the different N concentrations of the shoots were caused by the different free amino acid concentrations of the leaves.

For sulphur concentrations in roots, shoots and total plant no impact of nutrient heterogeneity could be detected. But the nutrient status of the plants was recognizable in the S concentrations, because plants with a nutrient supply adapted to 1.5*RGR had higher concentrations compared to the 0.5*RGR experiment.

Although the rate of photosynthesis, chlorophyll concentration and TNC concentration were dependent of the plant's nutrient level, the C concentrations were nearly equal. For this parameter neither the nutrient heterogeneity nor the nutrient status of the plants had a strong impact. Therefore it was not surprising that C/S and C/N ratios reflected the concentrations of the different nutrients: for the C/S ratios only the nutrient status of the plant had an impact. In contrast, the C/N ratios reflected the nutrient heterogeneity as well as the different nutrition levels.

In table 4 the responses of all functional parameters to nutrient heterogeneity and status were summarised. In the 1.5*RGR as well as in the 0.5*RGR experiment unequal supply of nutrients had an impact on just one third of the parameters. Generally, plants with high or low nutrient addition functionally respond similar to nutrient heterogeneity. Nearly 90 % of the functional parameters were influenced by the nutrient status of the plants. Only the C

concentrations of the shoots and the total plant were not affected by the given amount of nutrients.

It is clear that the supplied amount of nutrients had a stronger impact on functional plant behaviour than their distribution.

Table 4: Summary of the impact of nutrient heterogeneity and nutrient level on plants. **1** = impact, 0 = no impact. Total number of functional parameters = 17.

		Impact of nutrient heterogeneity		Impact of nutrient level
		1.5*RGR	0.5*RGR	
Functional	Rate of photosynthesis	0	0	1
	Chlorophyll concentration	0	0	1
	Sugar and starch concentration leaves	0	0	1
	Sugar and starch concentration roots	0	0	1
	Total free amino acid concentration leaves	1	1	1
	C concentration root sides	0	0	1
	C concentration total root	1	0	1
	C concentration shoot	0	0	0
	C concentration total plant	0	0	0
	N concentration root sides	1	1	1
	N concentration total root	1	1	1
	N concentration shoot	1	1	1
	N concentration total plant	1	1	1
	S concentration root sides	0	0	1
	S concentration total root	0	0	1
	S concentration shoot	0	0	1
	S concentration total plant	0	0	1
% of total functional parameters		35.2	29.4	88.2

3.3 Summary of structural and functional parameters

The nutritional level of the plants had a big impact on the plant performance. Biomass of the shoots were reduced in the 0.5*RGR experiment because of a reduced number of leaves, which were in addition smaller than those in the high nutrition experiment. These smaller leaves had less chlorophyll concentrations. The reduced shoot biomass caused a smaller shoot/root ratio in the low nutrition experiment. The lack of nutrients also influenced the rate of photosynthesis. The sugar and starch concentrations in the leaves as well as in the roots were higher in the plants with a low nutrient supply. But the C concentrations were nearly equal in both experiments. The free amino acid concentration in the leaves of the 0.5*RGR experiment was lower than in the high nutrition experiment. The given amounts of N and S were reflected in the plants: plants with a high nutrient supply had high N as well as S concentrations.

Plants responded to nutrient heterogeneity with different root biomass depending on their nutritional status. If plants responded to nutrient heterogeneity with different root biomass,

then this response was not changed by a further increase of heterogeneity. Higher root biomass at the root sides with higher nutrient concentration had increased root surfaces and lengths. Although the total root biomass was similar in the experiments, the N concentrations in the shoots increased with rising nutrient heterogeneity. But the higher nitrogen concentrations in the shoots did not cause more or bigger leaves with higher chlorophyll concentration and higher rate of photosynthesis. Only the free amino acid concentration in the leaves was increased with risen heterogeneity. In contrast to the N concentrations, the S concentrations in the shoots and in the roots were not influenced by the heterogeneous nutrient supply.

At the structural level nutrient heterogeneity had a local effect which was influenced by the nutrition level of the plants. On the other hand, at the functional level nutrient heterogeneity affected the total plant. This response was independent of the nutrition status of the plants.

In table 5 the impact of nutrient heterogeneity and nutrient level on all parameters was summarised. Altogether 29 parameters were measured: 12 structural and 17 functional parameters (see Table 2, p. 34; Table 4, p. 54). For 31.0 % respectively 27.5 % of all parameters in the experiments a consequence of nutrient heterogeneity was detectable. But the degree of reaction on an unequal supply of nutrients must not be the same. The impact of plant nutritional status could be shown in 69 % of all parameters. It has to be noticed that in only 41.6 % of the structural but for 88.2 % of the functional parameters the impact of nutrient level could be detected.

Table 5: Summary of the impact of nutrient heterogeneity and nutrient level on structural and functional parameters. Total numbers of parameters = 29.

	Nutrient heterogeneity				Nutrient level	
	1.5*RGR		0.5*RGR			
	impact	no impact	impact	no impact	impact	no impact
Sum structural parameters	3	9	3	9	5	7
Sum functional parameters	6	11	5	12	15	2
Total	9	20	8	21	20	9
% of total parameters	31.0	68.9	27.5	72.4	69	31

4. Discussion

In contrast to all other experiments about the impact of nutrient heterogeneity on plants (see 1.2, p. 2), the newly developed experimental design described here made it possible to raise nutrient heterogeneity step by step without changing the total amount of supplied nutrients. Furthermore, structural characteristics of the soil were not changed. The total amount of nutrients was kept at 1.5 or 0.5 of relative growth rate throughout the duration of the experiment. Therefore it was possible to measure the influence of heterogeneously distributed nutrients on maize plants under high and low nutritional status.

4.1 Development of the plants during the experiments

It was shown in this work that plants grown under heterogeneous nutrient conditions developed neither more nor healthier leaves during the experiments. On the other hand, number and average actual state of the leaves were influenced by the nutritional status of the plants. Plants adapted to 0.5*RGR developed less leaves, and these showed earlier senescence than plants of the 1.5*RGR experiment (Figure 7c, d; p. 23). Senescence is a normal process in leaf aging (Mooney *et al.*, 1981) and is influenced by the nitrogen status of the plant. If sufficient nitrogen is supplied to the plant, leaf senescence proceeds more slowly than in plants with insufficient nitrogen supply (Ono *et al.*, 2001). An early start of senescence can be expected with a high availability of photo assimilates (Wingler *et al.*, 2006) or a low inorganic nutrient supply (Crafts-Brandner *et al.*; 1998 Ono *et al.*, 2001). In this work, with rising heterogeneity, the shoot nitrogen concentration increased (Figure 21c, d; p. 39), but there was no obvious impact on leaf senescence. Although plants had a significant higher nitrogen concentration in the 1.0/0.0 treatments, this difference caused no difference in senescence.

At the end of the experiments the measured leaf surfaces showed no impact of nutrient heterogeneity. But it could be shown that plants with a low nutrient supply (0.5*RGR) had smaller leaf areas than plants at high nutrition. Leaf areas of plants in the 0.5*RGR experiment were nearly reduced by 50 % (Figure 8; p. 24). If plants are supplied insufficient with nutrients the leaf areas remain small (Chapin, 1991). It is known for barley and maize that insufficient nitrogen supply reduces the leaf area as well as the leaf biomass by nearly 50 % (Tesarova & Natr, 1986; Zhao *et al.*, 2003). For maize plants it is known that phosphate deprivation causes a reduced number of leaves with smaller leaf areas compared to control plants (Mollier & Pellerin, 1999). The current work is in agreement with the past work in the literature.

4.2 Impact of heterogeneously supplied nutrients on biomass

The heterogeneous distribution of nutrients in this work had no impact on total biomass (Figure 9; p. 25), on shoot biomass (Figure 10; p. 25) and on total root biomass (Figure 11; p. 26). The different nutritional status of the plants caused differences in shoot and total plant biomass. Plants of the 0.5*RGR experiment had a lower above ground biomass compared to plants of the 1.5*RGR experiment, because of the reduced number of leaves. The total root biomass did not reflect the supplied total amount of nutrients, adapted to 0.5 and 1.5 of the relative growth rate. Generally, an increase of nitrogen in the plant causes an increase in biomass (Imsande & Touraine, 1994). In the current experiments not only the nitrogen concentration was adapted to 0.5 or 1.5 of RGR but also all other nutrients were supplied in a constant relationship to nitrogen (see 2.5.2; p. 9). The results of this study reflected in this sense the experimental design: plants supplied with lower nutrient concentration had lower biomass than the others. In contrast to the total biomass, the nitrogen concentrations in the plants were influenced by the heterogeneous nutrient supply (Figure 21c, d; p. 37). The observed change of nitrogen concentration in the plants possibly did not cause a change of biomass, because of the low light conditions in the experiments. Low light intensities limit the rate of photosynthesis. This has a stronger impact on root- than on shoot growth (Marschner, 1995a). For example it is shown for *Pisum sativum* (L.) that at low light intensities ($190 \mu\text{mol m}^{-2} \text{s}^{-1}$) the plants develop only the half of the shoot biomass and the root biomass are reduced by 2/3 compared to plants at high light intensities ($390 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Reinhard *et al.*, 1993). It could not be excluded that the plant biomass in the experiments were influenced by light intensity because the light intensity was low in the experiments ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$). In further experiments with nutrient heterogeneity the possible influence of the light intensity on the biomass parameter needs to be examined.

In this work nutrient heterogeneity had no impact on shoot/root ratio (Figure 14; p. 29). But for *Glechoma hederacea* (L.) it is demonstrated that heterogeneous distribution of compost change the shoot/root ratio (Wijesinghe & Hutchings, 1999). The shoot/root ratio decreases with increase in heterogeneity. Unfortunately, the heterogeneity is made with compost, so that not only the nutrients but also the composition of micro organisms, soil density or water content is changed. The response of the plants cannot be explicit linked to nutrient heterogeneity, especially since it is known that micro organisms like protozoa can cause enhanced root proliferation (Bonkowski & Brandt, 2002). However, it is shown in other experiments in the literature, that the shoot/root ratio of maize plants is not influenced by nutrient heterogeneity which is made of nutrient solution. Neither a local enhanced concentration of phosphate or calcium have an impact on shoot/root ratio (Stryker *et al.*, 1974; Dejager, 1982) nor a local enhanced concentration of nitrate or potassium (Dejager,

1982). In the current work all nutrients were supplied heterogeneously and, as described in literature before, their distribution had no impact on shoot/root ratio.

In the current experiments it was shown that plants with insufficient nutrition (0.5*RGR experiment) had a smaller shoot/root ratio than plants with a high nutrient supply (Figure 14; p. 29). Plants at nutrient deprivation have a smaller shoot/root ratio because the biomass of the shoot is smaller compared to the root biomass (Brouwer, 1962; Wilson, 1988; Ericsson, 1995; Halsted & Lynch, 1996). This effect has been shown for a lot of different nutrients such as for example N (Marschner *et al.*, 1996) or P (Mollier & Pellerin, 1999). Unfortunately it was not possible with the current results to assess which of the reduced supplied nutrients caused the reduced shoot biomass and therefore smaller shoot/root ratio.

It was possible, however, to show for the first time that plants grown under high nutrient conditions responded to a smaller unequal nutrient supply (treatment 0.6/0.4) than 0.5*RGR plants (treatment 0.7/0.3) (Figure 12; p. 27). Root proliferation occurred on the split root sides supplied with more nutrients. Associated with root proliferation was an increased root surface as well as root length (Figure 15; 16; p. 30, 31) but the root volume was constant (Figure 17; p. 32). Because the total root biomass (Figure 11; p. 26) and total root surfaces, -lengths and volumes were equal between the treatments, the increase of these parameters on the nutrient rich split root side was associated with the decrease of root biomass at the nutrient poor split root side. In many other experiments in the literature it has been shown for different plants that root proliferation takes place as a response to nutrient heterogeneity (Fitter, 1994; Robinson, 1994; Hodge, 2004). That maize plants respond with root proliferation to localized fertiliser supply is first demonstrated in the middle of the 19th century (Nobbe, 1862). Later, the impact of local enhanced concentrations of single nutrients (e.g. NO_3^- , PO_4^- , K^+) on root growth is measured. Maize plants respond to locally increased phosphate concentration with a higher root growth (Stryker *et al.*, 1974) as well as for calcium and nitrate (Dejager, 1982). A local high concentration of nitrate causes an increased root density and growth of lateral branches (Granato & Raper, 1989). Localized high concentrations of sulphate and potassium cause no significant effects in maize roots (Dejager, 1982). But also for barley plants it is demonstrated in laboratory experiments that root proliferation occurs in local enhanced concentrations of phosphate as well as of nitrate. Roots show more first and second order laterals per unit root length and a great lateral root extension. A local increased concentration of potassium does not alter the root architecture (Drew *et al.*, 1973; Drew, 1975a). In this work all supplied nutrients were heterogeneously distributed so that it was not possible to assign the changes in root architecture to a single nutrient.

It could be shown for the first time that the plant response of root proliferation to nutrient heterogeneity depended on the nutritional status of the plant. Plants under high nutrient

conditions responded at the 0.6/0.4 treatment, plants under low nutrient condition responded at the 0.7/0.3 treatment (Figure 12; p. 27). But why did plants respond more sensitively under high nutrient conditions to nutrient heterogeneity than plants with an insufficient nutrient supply? The ratio of supplied nutrients between the left and the right root sides was not responsible for this effect, because the plants did not respond at the same treatment of heterogeneity. But the plants responded in both experiments and therefore there must be a common ground which induced root proliferation in the different treatments of the experiments. A possible explanation of the earlier response of the plants adapted to 1.5*RGR and the later response of the plants under low nutrient condition could be the concentration difference of the supplied nutrient solution. This concentration difference probably influenced the different response of the treatments. All nutrients were heterogeneously distributed in the experiments and the nutrients of the given solutions were calculated on the basis of nitrogen (see 2.5.2, p. 9). Nitrogen was supplied as nitrate to the plants in the experiments. Nitrate had the highest concentrations of all given nutrients. The other supplied nutrients were in a constant relation to it. Furthermore it is known that maize plants respond to spatially heterogeneous nitrate supply (Dejager, 1982; Granato & Raper, 1989). Therefore, the differences of nitrate concentrations of the 0.6/0.4 treatment in the high nutrition experiment were calculated for the whole experimental period. The smallest difference during the period (0.002 mmol/l) could be the possible threshold at which plants responded. This threshold was found in all treatments of the 0.5*RGR experiment except of the control. Plants in the 0.6/0.4 treatment did not respond to the nutrient heterogeneity although the threshold was exceeded for 4 days. But in the 0.7/0.3 treatment this threshold was exceeded for 7 days and the plants responded to an unequal nutrient supply. It is known from literature that plants need a certain time to respond to a local increased nutrient concentration with root proliferation. In different experiments maize plants as well as wheat need 5 days to respond (Granato & Raper, 1989; Sattelmacher & Thoms, 1995; vanVuuren *et al.*, 1996). In the available work the threshold in the 0.6/0.4 treatment of low nutrition experiment was apparently not exceeded long enough so that plants were able to develop significantly different root masses. In further research this threshold hypothesis needs to be examined.

4.3 Impact of nutrient heterogeneity on the internal nutrient concentration of the plants

4.3.1 Carbon

In the current experiments of this work neither the heterogeneous distribution of the nutrients nor the different nutritional status of the plants caused significantly different carbon concentrations (Figure 20a, b; p. 37). The values of the carbon concentrations included all carbon containing substances. Carbon was fixed in photosynthesis. It was not possible to

control the CO₂ content in the air during the experiments. In the 0.5*RGR experiment the result of the selective measurements of photosynthesis was lower than in the 1.5*RGR experiment (Figure 18; p. 35) but the plants with a limited nutrition had slightly enhanced C concentrations (Figure 20a, b; p. 37). To explain the differences between the experiments, research on plant carbon balancing is necessary.

4.3.2 Nitrogen

In this study the nitrogen concentration in different parts of the plants was measured. In the roots the nitrogen concentration reflected the heterogeneous distribution of N. In contrast to the sulphur concentration, the nitrogen concentrations were higher on the root sides where more nitrogen was supplied (Figure 23c, d; p. 43). Furthermore, it could be shown for the first time, that irrespective of the nutritional status of the plants (0.5, 1.5*RGR) the nitrogen concentration in the above ground part of the plant increased with increasing heterogeneity (Figure 21c, d; p. 39), while such an increase was not detectable for the also heterogeneously supplied sulphate. In other publications it is demonstrated for different plants and different nutrients that high local concentration of different nutrients causes an increased uptake of the respective nutrient (Drew & Saker, 1975; Drew & Saker, 1978; Burns, 1991; Sattelmacher & Thoms, 1995; vanVuuren *et al.*, 1996). An increased uptake of N and P from a local high supply of these nutrients has been demonstrated for barley. The ¹⁵N-nitrate uptake is enhanced by a factor of ~2.5 (Drew & Saker, 1975) and ³²P-phosphate uptake by a factor of ~2.5 - 5.0 compared to control treatments (Drew & Saker, 1978). For *Lactuca sativa* (L.) is demonstrated that 12 d after reduction from 100 % to ca. 10 % of the root system which is supplied with nitrate, the enhanced N uptake and development of new roots in the "patch", the condition of the control plants are restored (Burns, 1991). For nitrate and sulphate it is known that plants have different uptake systems: a high and a low affinity transport system (Smith *et al.*, 2000; Orsel *et al.*, 2002). For nitrate the existence of 3 different nitrate uptake systems has been demonstrated (Forde & Clarkson, 1999; Glass, 2002). If the external nitrate concentration is high (250 µM – 50 mM) a low-affinity transport system (LATS) is operating. At low external nitrate concentrations (0 - 0.5 mM) two high affinity transport systems are active. One of these is constitutive (Quaggiotti *et al.*, 2003)(K_m = 6 – 20 µM) whereas the other is induced by nitrate (K_m = 20 – 100 µM) (Crawford, 1995; Crawford & Glass, 1998). Furthermore it is known that the nitrate uptake systems are expressed in the total root (Touraine, 2004). A possible explanation for the different N concentration in the roots of the split root sides of this study could be the different transport systems. Because in maize plants nitrate assimilation occurred in the leaves (Andrews, 1986), most of nitrate taken up was transported to the above ground part of the plant. This explained, why with increasing heterogeneity the N concentration in this part of the plant

increased, too. In summary, in the current study plants had a higher nitrate uptake if the nutrient supply was heterogeneously.

The plants in the current experiments had different levels of nitrogen concentration, depending on their nutritional status. Plants with a nutrient supply adapted to 1.5*RGR contained on average 3.1 ± 0.15 % N per g dry matter. In contrast to this plants from the 0.5*RGR experiment contained less N (1.3 ± 0.12 % N/g dry matter) (Figure 20c, d; p. 37). If maize plants are optimally fertilized, the N content in the leaves is about 3.5-5.0 % N/g leaf dry matter (Bergmann, 1993). Therefore, the nitrogen concentration in the plants of the 1.5*RGR experiment reached nearly this optimal range reported from the literature. That plants in the 0.5*RGR experiment showed a lack of nutrients was detectable by the low N concentrations.

4.3.3 Sulphur

Higher concentrations of supplied sulphate on one of the split root sides did not cause an increased sulphur concentration in the respective root biomass (Figure 27a, b; p. 47), although root biomass and root architecture were influenced by nutrient heterogeneity. Furthermore the sulphur concentration in the above ground part as well as in the total plant did not differ. The heterogeneous supply of sulphate in the different experiments was not detectable in the plants (Figure 24a, b; 25a, b; p. 44, 45). These results were the opposite to the measured N concentrations in the different parts of the plants (see 4.3.2, p. 60). For sulphate one can distinguish between a high affinity transporter ($K_m \approx 10 \mu\text{M}$) and a low affinity transporter ($K_m \approx 100 \mu\text{M}$) (Hell, 1997). The high affinity transport system is expressed only in the roots, whereas the low affinity transport system is expressed in leaves as well as in roots (Leustek & Saito, 1999). The affinities to sulphate of these two systems are different. Also different is their spatial distribution in the roots: it has been demonstrated for *Arabidopsis thaliana* that the low affinity transport system is only expressed in the vascular parenchyma and not in endodermal or cortical cells (Takahashi *et al.*, 1997). The high affinity transport system mediates the sulphate uptake from soil into the plant (Leustek & Saito, 1999). The sulphur concentration of the roots at the different split root sides determined in this study can be explained with the inability of the high affinity transport system to use the local supply of sulphate. Additionally, for support of the hypothesis that the high affinity transporters were not able to use the local enhanced supply of sulphate, is the fact that sulphur assimilation occurs mostly in the leaves (Tabe & Droux, 2001). If there would be an enhanced uptake of sulphate at one root side, then the sulphur concentration in the above ground part of the plant would be higher than in the control treatment. This was not detectable.

Plants which were adapted to 1.5*RGR got higher amounts of sulphate than plants of the 0.5*RGR experiment. Plants with a low sulphate supply showed low sulphur concentrations

(Figure 24; p. 44). The different levels of sulphur concentrations in the plants depended on the supplied amount of sulphate.

4.4 Regulation of root proliferation

It was striking that in the experiments, if plants responded to nutrient heterogeneity at the level of root biomass, this response was independent of the increase (0.6, 0.7, ..., 1.0) or decrease (0.4, ..., 0.0) of the supplied nutrient concentration within the experiment. Roots are a "source" for the plant, because nutrient uptake occurs in the roots, and a "sink" for photo assimilates (Wissuwa *et al.*, 2005). It is shown for maize plants that a local high concentration of nitrate causes root proliferation and an increased root respiration compared to control experiments (Granato & Raper, 1989; Sattelmacher & Thoms, 1995). Furthermore, nitrate uptake is enhanced. However, until now it is not known whether root proliferation takes place because of high root respiration. An argument for such a respiration hypothesis is that root proliferation occurs, if the root temperature is locally increased (Sattelmacher & Thoms, 1995). An argument against this hypothesis can be, that a local high concentration of potassium increases K uptake and root respiration (Thoms, 1992) but does not cause root proliferation (Drew *et al.*, 1973; Drew, 1975a; Dejager, 1982; Thoms, 1992). If root proliferation as an answer to local high nutrient concentration is not regulated by root respiration, the regulation could occur locally in the roots or systemically in the shoots (Forde & Lorenzo, 2001). Unfortunately, the data of the current study did not allow to conclude anything about the location of regulation or the role of respiration. Root proliferation was independent of the total supplied nitrate as well as nitrogen concentration in the plant because in the experiments different total amounts of nitrate were supplied (see 2.5.2, p. 9) and different amounts of nitrogen were recovered (Figure 20c, d; p. 37). At the end of the experiments root biomass was nearly equal. It could be assumed that the amount of photo assimilates were necessary to develop the root biomass were nearly equal.

4.5 Effect of nutrient heterogeneity on photosynthesis and chlorophyll concentration

4.5.1 Photosynthesis

An impact of nutrient heterogeneity on the rate of photosynthesis could not be detected in this work (Figure 18; p. 35). It is known from literature that high local concentrations of nitrate, phosphate or potassium cause higher root respiration (Granato & Raper, 1989; Thoms, 1992; Sattelmacher & Thoms, 1995) but no publications about the impact of nutrient heterogeneity on the rate of photosynthesis are available. Therefore the results of the current study are new. For further research one would need to examine why a heterogeneous supply of nutrient had no impact on the rate of photosynthesis.

Plants with an insufficient nutrient supply (0.5*RGR experiment) showed a lower rate of photosynthesis than well fertilized plants (Figure 18; p. 35). From other experiments it is known that insufficient supply of nutrients to the plants reduces the photosynthesis rate (Natr, 1992). A lack of nitrogen affects the structure and the composition of the photosynthetic apparatus. A limitation of nitrogen causes in maize plants a reduction of the photosynthetic capacity (Khamis *et al.*, 1990b). Phosphate deprivation in maize plants causes lower photosynthesis rates (Khamis *et al.*, 1990a). Furthermore a lack of K, Mn, Fe or Cu reduces the rate of photosynthesis, too. In my study the lower rate of photosynthesis in the 0.5*RGR experiment could not be ascribed to a single nutrient, because all nutrients were adapted to the half of the relative growth rate.

4.5.2 Chlorophyll

Nutrient heterogeneity had no impact on the chlorophyll concentration of the leaves. No data about nutrient heterogeneity and chlorophyll concentration are known from other experiments in literature. Therefore the results of this work were new und needed to be examined with additional experiments.

It could be demonstrated in the available work that plants with insufficient nutrient supply had lower chlorophyll concentration than plants under high nutrition conditions (Figure 19; p. 36). The chlorophyll content in leaves correlated with the nitrogen nutrition. An increase of the nitrogen content in the plant is coupled with an increase of chlorophyll (Khamis *et al.*, 1990b; Ercoli *et al.*, 1993; Zhao *et al.*, 2003; Hirel *et al.*, 2005). But nitrogen is not the only factor which influences the chlorophyll content in the leaves. A deprivation of phosphate as well as a lack of manganese cause lower chlorophyll concentrations in the leaves (Khamis *et al.*, 1990a; Natr, 1992). These results agree with the lower rate of photosynthesis found during my study.

4.6 Impact of heterogeneously distributed nutrients on metabolites in the plants

4.6.1 Sugar and starch

An impact of nutrient heterogeneity on the sugar and starch concentrations in the leaves as well as in the roots was not detectable (Figure 28; 29; p. 48, 49). As already shown for the parameters photosynthesis and chlorophyll concentration, there were no reference values in the literature. Why nutrient heterogeneity had no impact on the sugar and starch concentrations needs to be examined in further experiments.

But with the available data from this study, it was possible to determine an impact of the different nutritional conditions on the sugar and starch concentrations (Figure 28; 29; p. 48, 49). Plants which were limited in nutrient supply showed significant higher TNC concentration in the leaves and in the roots (Figure 30; p. 50). Furthermore, plants with an insufficient

nutrient supply (0.5*RGR) had higher starch concentrations in the leaves than plants under high nutrient conditions (Figure 31; p. 51). Insufficient nitrogen nutrition in plants can cause an accumulation of sugars because less amino acids and proteins are built (Scheible *et al.*, 1997; Paul & Driscoll, 1997; Wingler *et al.*, 2006). The availability of phosphate is relevant for forming sucrose and starch in the chloroplasts. Phosphate deprivation causes higher starch concentrations in plants (Ariovich & Cresswell, 1983; Khamis *et al.*, 1990a). In field experiments with maize plants at different high nitrogen nutrition, starch concentrations from 180 – 230 $\mu\text{mol/g}$ (dry matter) are determined (Hirel *et al.*, 2005). For rice plants with different levels of phosphate nutrition it is possible to detect higher sugar and starch concentrations in the leaves at low light intensities than at high light intensities (Wissuwa *et al.*, 2005). It could be summarized that in this study the starch concentrations were lower than the known concentrations from literature. This could be caused by the low light intensities ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$) in the experiments of this work.

4.6.2 Free amino acids

The results of the current study showed no biomass change due to nutrient heterogeneity, neither for the shoots nor for the total roots or plants (Figure 10; 11; p. 25, 26), although the nitrogen concentrations increased with rising heterogeneity in the shoots and total plants. Because these increased nitrogen concentrations did not alter biomass, the internal nitrogen pool had to be increased. This is the first time the impact of nutrient heterogeneity on the amino acid concentrations in the leaves has been demonstrated (Table 3; p. 52). In further research the impact of heterogeneous distributed nutrients on the amount and quality of proteins in the leaves should be measured.

The total amino acid concentrations were higher in plants at high nutrient conditions in this work (Table 3; p. 52). Higher concentrations of alanine, glutamate and aspartate (80 – 86 % of the total amino acid concentration) were determined independently from the nutritional status of the plants. It has been demonstrated in field experiments for maize plants, that the amounts of free amino acids and proteins are higher in plants with a sufficient nitrogen supply compared to plants with insufficient nitrogen nutrition (Hirel *et al.*, 2005). Generally, high concentrations of alanine in C_4 plants have been detected (Heldt, 2003). In other experiments on maize plants in the vegetative phase of life, a high amount of alanine, glutamate and aspartate is measured in the leaves (Khamis *et al.*, 1990b; Hirel *et al.*, 2005). While glutamate is one of the important molecules for transporting nitrogen in plants (Lohaus *et al.*, 1998), aspartate is essential for building the amino acids asparagine, lysine, threonine and methionine (Heldt, 2003). The impact of the supplied amounts of nutrients on amino acid concentrations in leaves was confirmed in this available work.

4.7 Conclusion

As already mentioned in the introduction, plants are living in a heterogeneous environment and as demonstrated in this work, they are able to respond to this heterogeneity. Nutrient heterogeneity is defined in the current study as functional and as spatial heterogeneity, which is constant over time.

- The amounts of supplied nutrients were adapted to 0.5 as well as to 1.5 times of relative growth rate. That the amount of supplied nutrients was an important factor in the development of structures and functions of the plant was expected and could be demonstrated on the structural and on the functional level of the plants in this work.
- The amount of supplied nutrients had a stronger impact on plant behaviour than their distribution.
- Maize plants responded functionally to nutrient heterogeneity. One functional response was the increase of the nitrogen concentration with rising nutrient heterogeneity in the above ground part of the plants as well as at the total plant level. This was independent of the nutritional status of the plants. Another important functional response to heterogeneously distributed nutrients was a change in amino acid concentrations in the leaves which was higher under heterogeneous conditions. This functional response was also independent of the nutrient status of the plants.
- Maize plants responded structurally to nutrient heterogeneity. In dependency of the nutritional status of the plants, the structural response occurred locally at the root biomass level. Plants with a high nutrient addition rate responded earlier (0.6/0.4 treatment) than plants with a lack of nutrients (0.7/0.3 treatment). The ratios of supplied nutrients were probably not responsible for this response, because the response was possibly caused by a minimum difference of supplied nitrate concentrations in the split root sides within the treatments. If this threshold was exceeded long enough, plants responded with root proliferation. Furthermore, if plants responded to nutrient heterogeneity with developing roots, then this response was not influenced by a further rise of heterogeneity.

4.8 Outlook

In this study many facets of the impact of nutrient heterogeneity on maize plants were demonstrated and this given rise to new questions.

One of the main questions for further research is how the parameters biomass and primary metabolism are influenced by high light intensity.

All nutrients were supplied heterogeneously. In further research only one single nutrient (e.g. nitrate) needs to be heterogeneously distributed in order to test the correctness of the threshold hypothesis.

At the end of the experiments the plant internal nutrient (N, S) concentration was determined.

But when is the nutrient uptake rate affected under nutrient heterogeneity?

It was possible to demonstrate the impact of nutrient heterogeneity on the free amino acid concentrations in the leaves. In further research the impact of unequal nutrient supply on proteins or other nitrogen containing compounds in the leaves has to be checked.

Plants responded with root proliferation to nutrient heterogeneity. More research about the regulation of this response is necessary.

The experiments of this work were done with maize plants. In order to test the general validity of the conclusion, more experiments with different plant species need to be done.

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