The influence of spatially heterogeneous soil temperatures on plant structure and function

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Contents

Abstract

In nature, vertical gradients in soil temperature are ubiquitous, but research on the influence of spatially heterogeneous soil temperatures on plant structure and function is scarce. Most experiments with plants even ignore the gradient in soil temperature found under natural conditions. For this reason, in this study it was examined for the first time, whether a vertical gradient in soil temperature influences plant growth and development in a different way than uniform root temperatures usually examined in the literature. Furthermore, it was analyzed whether functional and/or structural traits of the plant might be responsible for these potential effects. Data of barley plants (*Hordeum vulgare* cv. Barke) grown at a vertical root temperature gradient (RTG) of 20-10°C from the top to the bottom of a plant pot were compared with data obtained for barley plants grown at uniform root temperatures (RT) of 10°C, 15°C and 20°C, respectively.

Plants grown at the RTG developed faster and produced more biomass compared to plants grown at uniform root temperatures. The root system was characterized by shallow rooting with most roots present in 0-10 cm depth and a quite high fraction of thick roots (≥ 1.0 mm in diameter) in the entire root system. In this way, the root system of plants grown at the RTG was similar to plants grown at 15°C RT. However in contrast to 15°C RT, plants grown at 20-10°C RTG did not reach highest fraction of total root length in 0-5 cm but in 5-10 cm depth, although less root dry weight was present in 5-10 cm compared to 0-5 cm depth at both temperature treatments. This was explained by differences in fractions of individual root diameters within the respective depths. Additionally, experiments on N metabolism in plants revealed higher concentrations of most free amino acids in shoots at 20-10°C RTG and varying protein concentrations in roots between plants grown at RTG and 15°C uniform root temperature. Therefore, it was demonstrated that a vertical gradient in root temperature influences plant structure and function in a different way than the respective uniform root temperature representing the average temperature of this gradient.

No significant differences between 20-10°C RTG and 15°C RT occurred, when nutrient uptake and translocation were analyzed with stable isotopes as tracers (^{15}N, ^{25}Mg). However, in general it has to be stated that at active nutrient uptake processes direct root temperature effects, e.g. lower N uptake at 10°C RT compared to higher root temperatures were overridden by the adaptation of plant structure to the respective root temperature. This underlines the importance of structural traits (e.g. biomass allocation to the shoot, fractions of individual root diameters) to nutrient demand and supply. In contrast, direct temperature effects remained detectable at passive uptake processes (e.g. Mg). Therefore, it was hypothesized, that plants grown at a vertical root temperature gradient grow faster compared to plants at uniform root temperatures due to a combination of structural and functional components making nutrient uptake, translocation and use more effective.

Furthermore, it was shown, that root temperature effects on plant structure change in amplitude with plant age and development stage. Consequently, effects found in this study represent a snapshot of plant responses to root temperature.

Zusammenfassung

Unter natürlichen Bedingungen liegen stets vertikale Temperaturgradienten im Boden vor. Ihr Einfluss auf die Pflanzenstruktur und -funktion ist jedoch nur wenig erforscht und ihre Existenz wurde in Experimenten mit intakten Pflanzen bisher weitestgehend vernachlässigt. Daher wurde in dieser Arbeit erstmals untersucht, ob sich ein vertikaler Temperaturgradient im Boden anders auf das Pflanzenwachstum und die -entwicklung auswirkt als homogene Bodentemperaturen, die üblicherweise in der Literatur betrachtet werden. Des Weiteren wurde analysiert, inwiefern funktionelle und/oder strukturelle Eigenschaften der Pflanzen für die möglicherweise auftretenden Effekte verantwortlich sind. Daten von Gerste-Pflanzen (*Hordeum vulgare*, Var. Barke), die bei einem vertikalen Wurzeltemperaturgradienten (WTG) von 20-10°C von oben nach unten im Pflanzentopf gewachsen sind, wurden mit Daten von Gerste verglichen, die bei homogenen Wurzeltemperaturen (WT) von 10°C, 15°C bzw. 20°C gewachsen ist.

Bei dem WTG entwickelten sich die Pflanzen schneller und bildeten mehr Biomasse aus als Pflanzen, die bei homogenen Wurzeltemperaturen wuchsen. Die Wurzeln konzentrierten sich in den obersten 10 cm des Pflanzentopfes und das Wurzelsystem wurde von einem relativ großen Anteil dicker Wurzeln gebildet (≥ 1.0 mm Durchmesser). In dieser Hinsicht ähnelte das Wurzelsystem der Pflanzen bei 20-10°C WTG dem der Pflanzen bei 15°C WT. Allerdings fand sich, im Gegensatz zu 15°C WT, bei 20-10°C WTG der höchste Anteil an der Gesamtwurzellänge nicht in 0-5 cm sondern in 5-10 cm Tiefe, obwohl bei beiden Temperaturen die Wurzelmasse in 5-10 cm Tiefe geringer war als in 0-5 cm. Dies konnte auf Unterschiede zwischen den Anteilen der einzelnen Wurzeldurchmesser in den jeweiligen Substrattiefen zurückgeführt werden. Außerdem zeigte die Untersuchung des N-Metabolismus, dass die Konzentration der meisten freien Aminosäuren im Spross höher und ein Unterschied in der Proteinkonzentration in Wurzeln erkennbar war, wenn man Pflanzen bei 20-10°C WTG und bei 15°C WT miteinander verglich. Diese Ergebnisse zeigen, dass die Pflanzestruktur und -funktion von einem vertikaler Temperaturgradient im Wurzelraum anders beeinflusst wird, als von der, dem Mittelwert des Gradienten entsprechenden, homogenen Wurzeltemperatur.

Die Untersuchung der Nährstoffaufnahme und -translokation mit Hilfe stabiler Isotope (^{15}N, ^{25}Mg) zeigte keine signifikanten Unterschiede zwischen 20-10°C WTG und 15°C WT. Insgesamt bleibt jedoch festzuhalten, dass bei aktiven Nährstoffaufnahmeprozessen direkte Wurzeltemperatureffekte, z.B. eine geringere N Aufnahme bei 10°C WT im Vergleich zu höheren Temperaturen, durch Anpassung der Pflanzenstruktur an die entsprechenden Wurzeltemperaturen überlagert wurden. Dies unterstreicht die Bedeutung struktureller Merkmale (z.B. Biomassen-Allokation zum Spross, Wurzeldurchmesseranteile) für die Nährstoffnachfrage und –versorgung. Bei passiver Nährstoffaufnahme (z.B. Mg) blieben die direkten Temperatureffekte hingegen erkennbar. Es wurde die Hypothese aufgestellt, dass das schnellere Wachstum der Pflanzen bei vertikalem Temperaturgradienten im Boden auf eine Kombination von strukturellen und funktionellen Komponenten, die die Effizienz von Nährstoffaufnahme, -translokation und -gebrauch steigern, zurückzuführen ist.

Außerdem konnte gezeigt werden, dass Temperatureffekte auf die Pflanzenstruktur je nach Alter und Entwicklungsstadium der Pflanze unterschiedlich ausgeprägt sind. Daher handelt es sich bei den in dieser Arbeit gezeigten Effekten um eine Momentaufnahme der Pflanzenreaktion auf die Wurzeltemperatur.

1. Introduction

Plants are sessile organisms and are exposed to temporally as well as spatially varying environmental conditions with changes in e.g. humidity, temperature and nutrient availability. While research is quite common on temporally changing conditions, most knowledge of plant response to environmental factors results from experimental studies conducted at spatially uniform conditions, when not carried out in the field. Especially information on the influence of spatially different soil temperatures on single plant behavior is scarce (cf. Sowinski *et al.*, 1998; Bland *et al.*, 1990; Mosher & Miller, 1972), whereas scientists have been aware of the possible influence of spatially heterogeneous nutrient availability on plant response for some time (cf. Hutchings & John, 2004; Rist, 2006). However, knowledge of plant responses to spatially heterogeneous soil temperatures is essential presuming as some plant responses only reveal under these patchy conditions (Hutchings & John, 2004). The detailed understanding of soil temperature effects on mechanisms involved in plant growth and development is necessary e.g. for modeling plant yield and carbon balance (Wu *et al.*, 2005) - also against the background of climate change (Gunn & Farrar, 1999; Long & Hutchin, 1991).

1.1 Soil temperature and its dynamics

Temperature is an important factor influencing most biological processes (e.g. Lambers *et al.*, 1998). Thus, it is essential to understand plant response to temporally and spatially changing temperatures, as this might ultimately result in significant differences in plant development and productivity (McMichael & Burke, 1998).

In general, soil temperature is related to air temperature. Both describe sinusoidal oscillations on diurnal and annual scale. This oscillation depends on the energy balance between incoming fluxes of short-wave (sun and atmosphere) and long-wave (sky) radiation and the fluxes of emitted long-wave (by soil) and reflected short-wave (albedo) radiation at the soil surface (Hillel, 1998). Therefore, on larger scale spatial disparities in temperature mainly depend on differing latitude and altitude of the plant habitats as well as on their inclination and exposition (Scheffer *et al.*, 2002).

However, depending on soil depth changes in soil temperature are delayed and lower in amplitude than the temperature variation aboveground. Temporal and spatial temperature changes are strongest within the uppermost 20 cm of soil, while only little change in soil temperature occurs below 40-60 cm soil depth during the day (Fig.1; Scheffer *et al.*, 2002).

Seasonal amplitude is strongly damped belowground and seasonal temperature extremes occur much more delayed below 40-60 cm depth compared to the upper soil layers (Fig. 2). Differences between soil and air temperature as well as in soil temperature with altering soil depth are due to soil properties (soil surface roughness, soil color, soil density and soil water content). Furthermore, differences in soil properties can locally alter soil temperature.

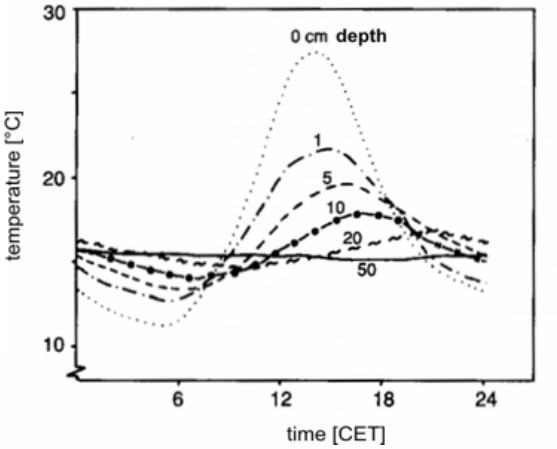

Fig. 1: Daily temperature changes depending on soil depth in sandy Cambisol at Worpswede in august (after Miess (1968) in Scheffer et al. (2002)).

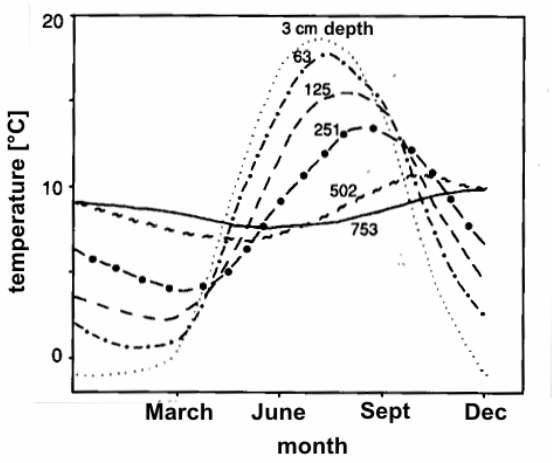

Fig. 2: Seasonal soil temperatures depending on depth; soil from Königsberg (after Schmidt & Leyst, in Geiger (1961), in Scheffer et al. (2002)).

Soil surface roughness, soil color, soil water content and soil density are the components controlling radiation absorption and emission properties of soils. Surface roughness and soil color determine the albedo of soil. The specific heat capacity (J cm^{-3} K^{-1}) of a soil, i.e. heat content per unit mass per unit change in temperature, as well as heat transfer from warm to colder regions within the soil are strongly related to bulk density, mineral composition and water content (cf. Ochsner *et al.*, 2001).

Convection and conduction are the two mechanisms controlling heat transfer within soil. Convection means heat transfer via a heat-carrying mass. For this reason, water content of the soil is most important for this movement as water is an excellent heat absorbent and is usually moving through the soil. In contrast, conduction occurs in soils at any time. According to

Fourier's law (Eqn. 1), heat flux in a homogeneous body is in direction of, and proportional to, the temperature gradient.

$$q_h = -\kappa \nabla T \qquad \textbf{Eqn. 1}$$

q_h = thermal flux (amount of heat conducted across a unit cross-sectional area in unit time)
κ = thermal conductivity
∇T = spatial gradient of temperature

However, composition of soils is seldom homogeneous and the thermal conductivity of specific soil constituents differs markedly. Therefore, van Bavel & Hillel (1976) used Eqn. 2 (based on de Vries, 1975) for determining thermal conductivity of an unsaturated soil:

$$\kappa_c = (f_w \kappa_w + k_s f_s \kappa_s + k_a f_a \kappa_a) / (f_w + k_s f_s + k_a f_a) \qquad \textbf{Eqn. 2}$$

κ_c = composite (soil) thermal conductivity
κ_w, κ_s, κ_a = thermal conductivity of water, solids (average value), air
f_w, f_s, f_a = volume fraction of water, solids, air
k_s, k_a = ratio between the space average of the temperature gradient in the solid relative to the water phase, the corresponding ratio for the gradients in the air and water phases

Nevertheless, heat transfer does not always imply immediately measurable soil temperature changes. This depends on the specific heat capacity of the soil and its variation with water content. The relation between temperature conductivity, heat conductivity and specific heat capacity in dependence on water content is shown in Fig. 3.

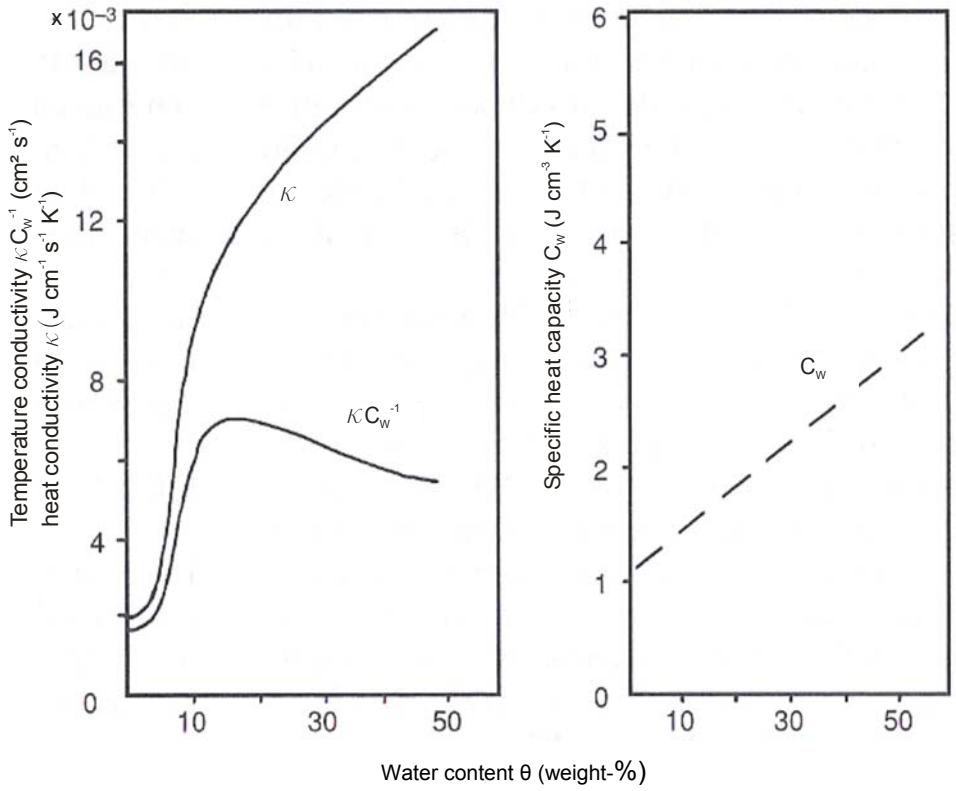

Fig. 3: Relation between heat conductivity, temperature conductivity and specific heat capacity of the soil in dependence on water content (after Bolt et al. (1965) in Scheffer *et al.*, 2002).

The mentioned traits and mechanisms determining temperature conductivity cause the previously mentioned vertical temperature gradients in each soil. Plant roots are exposed to them and therefore, the plant has to cope with them at any time. Additionally, as plants change the soil water content by water uptake, they also may influence soil temperature. Furthermore, plants can reduce evaporation and absorption of radiation by shading the ground (McMichael & Burke, 1998), and thus locally alter the soil temperature.

1.2 Plant response to soil temperature

In nature, plants can cope with a wide range of soil temperatures (further on synonymously used with “root temperature”) without showing damage. The bandwidth of temperatures, in which plant roots can grow, characterized by minimal, optimal and maximal temperatures, depends on the climate zone from which a plant species originated. For instance, root growth of plants from temperate regions has its optimum between 10°C and 30°C, but still continues around 0°C. In contrast, optimum temperature of subtropical species is higher, while root

growth already stops below 10-15°C (Bowen, 1991). Species-specific differences in growth optima occur within a climate zone. Lyr (1996) showed growth optima by measuring total biomass increment for European tree species ranging from about 15°C root temperature (e.g. *Picea abies*, *Larix deciduas*, *Betula verrucosa*) to 25°C (*Quercus robur* and *Carpinus betulus*) and 30°C (*Pinus nigra*).

In general, biomass increases with increasing root temperatures until the optimum temperature is reached (Matthews & Hayes, 1982; Clarkson *et al.*, 1986; DeLucia *et al.*, 1992). Within the optimum temperature range biomass allocation to roots is lower than to shoots. At sub- or supra-optimal temperatures allocation to the roots is favored (Equiza *et al.*, 2001; Davidson, 1969; Engels, 1994; Boucher *et al.*, 2001).

Root temperature does not only effect plant growth and biomass allocation, but also causes changes in plant development, morphology and physiology. A large variety of results on theses aspects has been reported in the literature, due to a huge diversity of experimental setups used and variables examined. Here, only some general remarks should be passed:

- Time until germination is reduced and plant development is accelerated by increasing root temperature (Beauchamp & Lathwell, 1967; Bowen, 1991).
- Elongation of individual roots (Abbas Al-Ani & Hay, 1983; Stone & Taylor, 1983; Ching & Barber, 1979) and root branching (McMichael & Quisenberry, 1993; Kaspar & Bland, 1992; Stone & Taylor, 1983; Brouwer & Hoogland, 1964) are positively correlated with increasing root temperature until the temperature optimum is reached.
- Declining root temperatures decrease stomatal conductance and photosynthesis (Starr *et al.*, 2004) by influencing the water status of the plants (Dodd *et al.*, 2000; Boucher *et al.*, 2001).
- Root temperature affects nutrient uptake and allocation in the plant. However the responses significantly differ depending on nutrient examined and nutrient status of the plant (Engels *et al.*, 1992; White *et al.*, 1987; Clarkson & Warner, 1979; Ching & Barber, 1979; Lee & Drew, 1986).

These results were obtained by experiments generally conducted at uniform root temperatures. However, Sowinski et al. (1998) were some of the few scientists focusing on the influence of spatially heterogeneous root temperatures on plant responses. They figured out, that an increase in soil temperature accelerates assimilate transport in corn. Furthermore, they stated that the extent to which a vertical soil temperature gradient influences the assimilate transport depends on the fraction of roots exposed to lower temperatures occurring in the temperature range of the gradient. However, no information was available about plant

development and growth when exposed to vertical soil temperature gradients right from the beginning of growth, since they used plants pre-grown at uniform air and soil temperatures (24°C) for several days.

However, mechanisms underlying the specific plant responses to root temperature are still not completely understood.

1.3 Challenges in research on plant-soil temperature interactions

The lack in information about the influence of spatially different soil temperatures on plant structure and function could be explained by difficulties in applying controlled, stable soil temperature gradients within the experimental setup and by general difficulties in determining temperature effects on plants:

Under natural conditions soil temperature interacts with other abiotic (e.g. soil water content, nutrient availability, soil density etc.) as well as biotic (e.g. microorganisms, mycorrhiza) components. Therefore, it is not easy to examine soil temperature effects and exclude effects caused by other factors (Pregitzer *et al.*, 2000). The influence of root temperature may decrease as other factors are more limiting (Brouwer, 1964). Furthermore, interactions with other factors can hide its impact (Boucher *et al.*, 2001) as well as interactions between other factors can mimic it (Forde & Lorenzo, 2001). Another challenge is to establish controlled root temperatures and to analyze responses of the intact root system.
To overcome these difficulties, most research was conducted with uniform root temperatures in highly artificial experimental setups (e.g. hydroponics, agar plates) (Loveys *et al.*, 2002; Engels *et al.*, 1992; Rufty *et al.*, 1981; Fortin & Poff, 1990). When research was conducted in solid media in the laboratory (e.g. DeLucia *et al.*, 1992; Equiza & Tognetti, 2001), mostly other abiotic factors beside soil temperature were not strictly controlled. Sometimes it was even not distinguished between root and shoot temperature (Garmash, 2005; Gunn & Farrar, 1999). When studies were conducted under natural conditions in the field, it was usually not possible to control the present vertical soil temperature gradient and to guarantee the absence of other factors than temperature determining plant responses (Fitter *et al.*, 1999).

However, the variety of experimental setups, exposure temperatures (chilling, temperate and heat-shock) and plant species used (annual crops, perennial herbaceous plants and trees)

enhance the difficulty to compare results available for plant responses on soil temperature. Even the duration of temperature treatment (Engels *et al.*, 1992; Brouwer, 1964) as well as pretreatment temperatures of the plants (White *et al.*, 1987; Brouwer, 1964; Clarkson & Warner, 1979) influence plant responses.

Furthermore, it is common practice in experiments to harvest plants grown at different root temperature treatments either at same age or at same development stage for analyzing root temperature effects (Cumbus & Nye, 1982; Beauchamp & Lathwell, 1967). Therefore it is difficult to decide, whether differences in plant responses were caused by root temperature or by the differing development stages respectively ages of the plants at harvest time. Furthermore, it is not known yet, whether plant responses to root temperature may also alter with changing plant age and development stage. A study is missing, which comprises both aspects at a time.

1.4 Aim of this study

As outlined before, research on spatially varying soil temperature is essential to understand plant response to soil temperature in nature. This research has to be conducted under controlled (all other abiotic factors constant) and in the range of natural conditions to avoid side-effects caused by the use of artificial setups. Furthermore, the obtained results have to be compared with those achieved by experiments with uniform soil temperatures. This allows answering the question, whether plants grown at a specific vertical gradient in root temperature respond in the same way as plants, which grow at the uniform root temperature representing the average temperature of the gradient. Furthermore, in this way the relevance of results obtained for research with uniform root temperatures could be assessed for plant responses to soil temperature at fluctuating environmental conditions.

Therefore, this study focused on measuring root temperature effects, i.e. effects caused by vertical temperature gradients as well as by uniform temperatures, on plant structure (e.g. biomass allocation, root morphology) and on plant function, particularly on nutrient uptake and translocation, under controlled and largely natural conditions. Nitrogen and magnesium were chosen as representatives for analyzing nutrient uptake, because they are essential for plant growth and allowed to examine two different uptake systems at a time. N uptake is an active process (e.g. Glass *et al.*, 1992), whereas Mg uptake is passive (e.g. Ferguson & Clarkson, 1976). Additionally, root temperature effects on some components of the N

metabolism (e.g. amino acids, proteins) should be analyzed due to their significance in plant life. For instance, it is already known that temperature influences e.g. membrane proteins in barley (Caldwell, 1987) and enzyme activities (e.g. glutamine synthetase, glutamate dehydrogenase) in leaves of soybean (Duke *et al.*, 1979).

In detail, the **first aim** of this study was to analyze, whether plant growth during the vegetative stage was altered by vertical soil temperature gradients when compared to plants grown at uniform soil temperatures.
To answer this question, (1) plant biomass as well as C and N allocation were measured at a certain plant age. (2) Developmental stages of the plant were monitored during growth.
The **second aim** was to figure out, (a) whether changes in plant structure and/or function occurred at vertical soil temperature gradients compared to uniform soil temperatures and (b) whether these might be responsible for possible differences in plant growth.
Therefore, (1) root architecture was monitored by determining e.g. root length and diameter composition (fraction of different root diameters on the entire root system) as well as its underlying variables “root elongation” and “branching”. (2) N fractionations (NO_3, free amino acids and proteins) were examined (3) Nutrient uptake and translocation (N and Mg) as well as nutrient uptake kinetics were studied by using stable isotopes (^{15}N and ^{25}Mg).
The **third aim** of this work was to determine, whether plant responses to soil temperature vary with plant age and development stage.
Structural characteristics (biomass, root morphological etc.) of plants grown at two uniform root temperatures were compared at (1) same age but different development stage, (2) different age but same development stage and (3) during plant aging.

2 Materials & Methods

2.1 Plant material

All experiments were conducted with spring barley (*Hordeum vulgare* cv. Barke; BSA-Nr.: 1582). *Hordeum vulgare* (family: Poaceae) is an important crop and scientifically well described. Barke developed out of cross breeding varieties Libelle x Alexis (Saatzucht Josef Breun GdbR, Herzogenaurach, Germany). It is a malting variety and was firstly launched in 1996. Today it exhibits worldwide relevance, because of constant high yield and quality capacity at most environmental conditions. In Germany it is mainly grown in northern Rhineland-Palatinate, Thuringia and Lower Saxony. Furthermore it has an excellent resistance against mildew and leaf rust (Weiß & Pechstein, 2005).

2.2 Growth conditions

The experiments were undertaken in the laboratory, as it is necessary to keep other environmental conditions constant, when examining the influence of root temperature on plant structure and function.

Light, air temperature and air humidity

Experiments were conducted in a cultivation room. Shoot temperature (22 ± 1°C) was constant over time and was maintained at all root temperature treatment. Light conditions were the same at all temperature treatments. Lamps (COOL WHITE, OSRAM L 36W/21-840, Lumilux Plus Eco) provided a photon flux density of 240-250 µmol m^{-2} s^{-1} at 30 cm above the substrate surface. The day/night light regime was 12/12 h. For logistical reasons, plants used in the *nutrient uptake kinetics* experiment (cf. Chap. 2.5) and in the *nutrient uptake in sand* experiment (Chap. 2.6) were grown at different light conditions until used in the experiments. Here, the photon flux density was about 100 µmol m^{-2} s^{-1} (OSRAM FLUORA 36/77). Air humidity in the cultivation room ranged between 40 – 60 % r.h.

Root temperature

Two different types of temperature boxes surrounding the plant roots were designed, because the effects of root temperature should be analyzed either at uniform root temperatures or at a vertical gradient in root temperature.

For stable uniform root temperatures over time, boxes (about 115 cm x 30 cm x 43 cm inner dimensions) were constructed out of 5 cm thick isolation material (Jackodur), were stabilized by PVC cover and had an outlet at one side. A uniform temperature of the entire root system was reached by a tube system (Gardena®) on the walls and floor of the box (Fig. **4**) and by two fans constantly circulating air and positioned in 40 cm distance from each end of the box. For uniform root temperature (RT) treatments the temperature of the root compartment was adjusted to 10°C, 15°C and 20°C, respectively. These root temperatures corresponded to temperature regimes in the field during the vegetative growth period of crops in the mid latitudes (Bowen, 1991; Briggs, 1978). The set temperature was reached in plant pots (cf. Chap. 2.3.1) ~ 7 h after positioning the pots inside the temperature box. Temperature difference between the individual plant pots was ± 0.6°C.

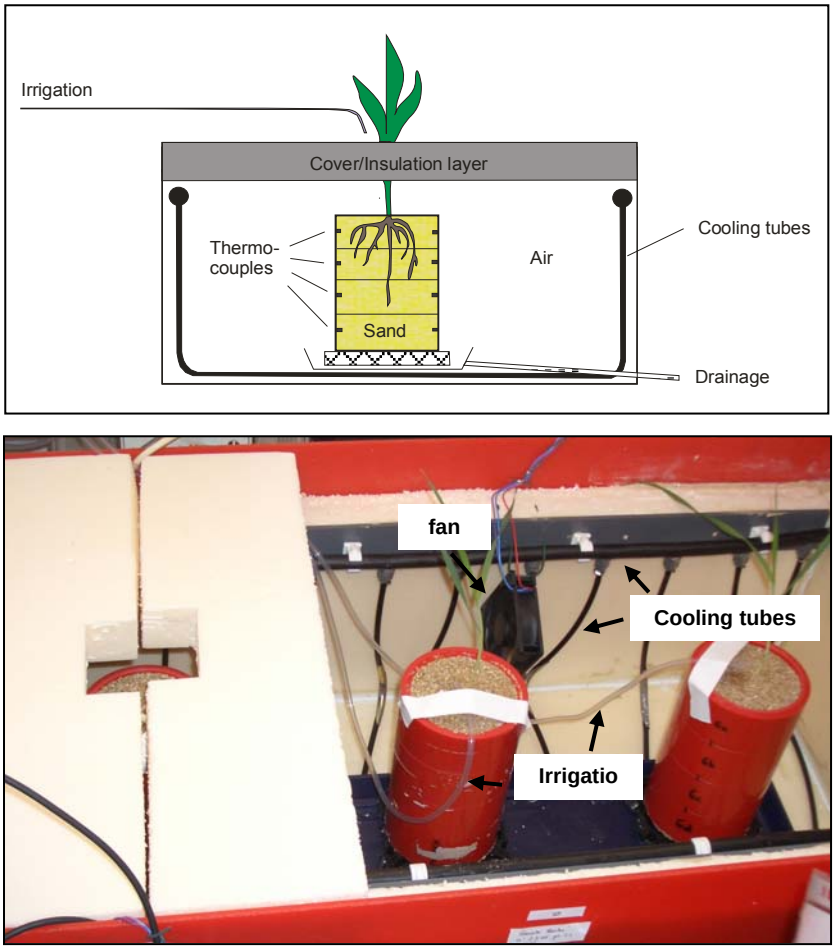

Fig. 4: Box system for adjusting uniform root temperatures.

Boxes of same dimension and material as already described for adjusting uniform root temperatures were used for adjusting the vertical gradient in root temperature (RTG). However, these boxes contained two separate tube systems: one at the top of the box and one at the bottom (Fig. 5). Each tube system ran at a different temperature. Between these two systems a stable temperature gradient was generated. In order to allow the gradient to form and maintain, the plant pots (c.f. Chap. 2.3.1) inside the box were surrounded by sand. Each plant pot had a separate outlet for drainage. A temperature of 20°C was set at the top of the plant pot and a temperature of 10°C at the bottom. The gradient developed over a distance of 20 cm with temperatures of in average 20°C, 16°C, 13°C and 10°C measured in 2.5 cm, 7.5 cm, 12.5 cm and 17.5 cm depth, respectively (Fig. 6). Experimental temperature in plant pots was reached after ~ 24 h and temperature difference between the pots was ± 1.0°C.

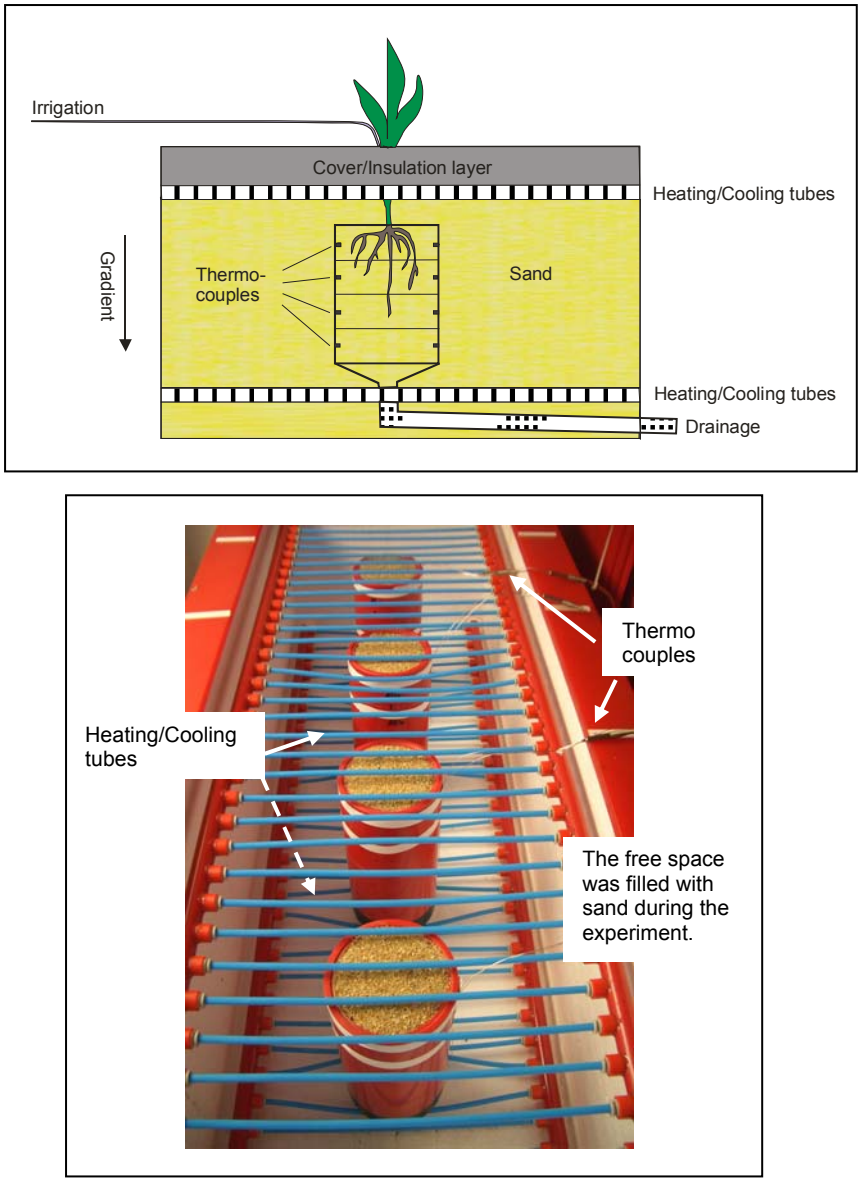

Fig. 5: Box system for adjusting a vertical gradient in root temperature.

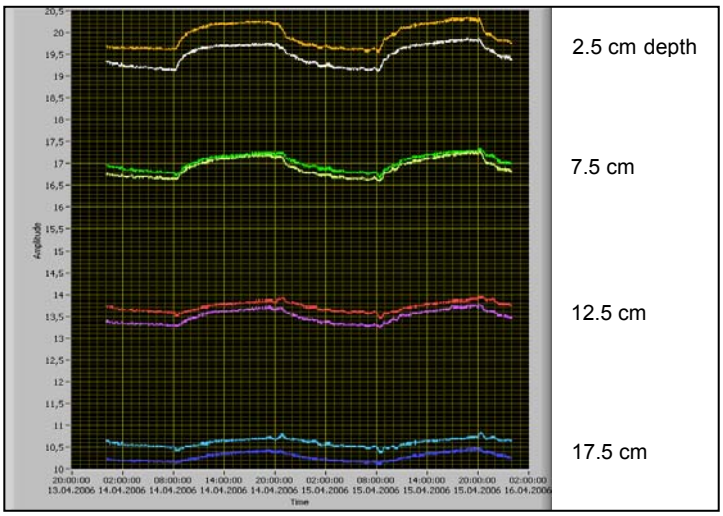

Fig. 6: Root temperature measurements shown for two plant pots simultaneously. Measurements were conducted in four depths. A time interval of two days is presented.

Julabo F32-MC appliances were used as cooling devices, containing a mixture of glycol and deionized water (1:1 v/v) as cooling medium. This cooling medium was continuously pumped through the tube systems of the boxes to maintain the respective experimental root temperatures. Temperature inside plant pots was measured by copper-constantan thermocouples (304 SS-Mantel, 0.5 mm; Omega Newport Electronics GmbH) positioned in the pots at four different depths (2.5 cm, 7.5 cm, 12.5 cm and 17.5 cm) and connected with a data logger (FieldPoint FP-1601 + FP-TC-120, National Instruments and labview 7.1 software, National Instruments). In total, 8 plants grown in plant pots (cf. Chap. 2.3.1) and 6 plants grown in rhizotrones (cf. Chap. 2.4.1) were simultaneously analyzed per temperature treatment (4 respectively 3 plants per temperature box).

2.3 Measurement of root temperature effects on plant structure

2.3.1 Growth system

Experiments for measuring root temperature effects on plant structure (e.g. biomass, root morphology) were conducted in specially designed plant pots. These plant pots were made of PVC and had a volume of about 1277 cm^3 and a height of 20 cm. At the bottom, a sieve tissue (250 µm mash size) prevented roots from leaving the pot but also allowed drainage. The option to divide the pots vertically into 4 segments - each of 5 cm height - allowed root analyses within different depths (Fig. 7).

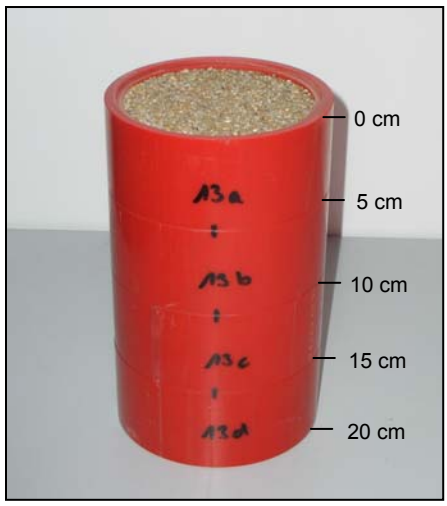

Fig. 7: Plant pot separable into four substrate layers, each of 5 cm height.

Washed and dried quartz sand (SiO_2: 95.7 weight-%) was used as substrate enabling the control of abiotic factors like substrate density, water content and nutrient content. This was possible, because in contrast to soil (1) sand is available in well defined grain size, (2) is poor in nutrients (adsorption capacity is very low), and (3) microorganism content is low even at non-sterile conditions due to low C sources. The defined grain size (here: 0.7 - 1.4 mm) of the sand allowed reproducible substrate densities by manually packing the plant pots. A medium density (AG Boden, 1996) typical for sandy soils was chosen (1.65 g cm^{-3}) (Scheffer *et al.*, 2002). This enabled a quite easy penetration of the substrate by roots and avoided water logging. Furthermore, stable water and nutrient contents and distributions within the substrate could be adjusted by a constant substrate density. A stable total water content of 10.4 weight-% on average was adjusted within the plant pots. However, gravity caused a gradient in water content. This gradient was stable and reached values of ~ 6 weight-%, 8 weight-%, 13 weight-% and 15 weight-% in 0-5 cm, 5-10 cm, 10-15 cm and 15-20 cm substrate depth, respectively. These stable water contents were achieved by saturating the plant pots from the bottom to the top until all air inside the substrate body was replaced by deionized water and by subsequent drainage for 48 h. Tops of the plant pots were closed by parafilm during drainage to avoid evaporation and thus drying of the uppermost substrate layer. Furthermore, the plant pots were positioned on a moist sand bed to avoid hanging menisci and thus unequal drainage. Continuously percolating drip irrigation (40 ml h^{-1}) by peristaltic pump (Minipuls 3, Gilson) was used, which started immediately after plant pots were positioned in the temperature boxes. The continuous irrigation system maintained stable water and nutrient contents as well as constant heat conductivity conditions (cf. Chap. 1.1) and therefore stable root temperatures. Consequently, the heating/cooling devices were able to

maintain stable root temperatures, even a stable vertical temperature gradient, although the added solution was not pre-cooled to the experimental temperatures.

To guarantee constant growth conditions in each plant pot, plants were directly germinated in the pots. Seeds were sown 1 cm below sand surface. Within the first days after germination barley seedlings get most nutrients from the grain. Therefore, irrigation switched from tab water to 0.5 modified Hoagland solution (2.5 mM KCl, 2.5 mM $NaNO_3$, 2.5 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, 0.5 mM KH_2PO_4, 0.5 mM trace elements ($MnCl_2$, $CuSO_4$, $ZnSO_4$, H_3BO_3, $NaMoO_4$) and 0.5 mM Fe-EDTA) only 9 days after germination.

2.3.2 Experimental design

Most research of root temperature effects on plant structure in the literature was conducted either with plants of same age or of same development stage, rarely taking into account both aspects. The present study contributes information about variation in plant responses to root temperature depending on these aspects by performing analyses on (1) plants of same age as well as on (2) plants of same development stage. Additionally, (3) changes in plant responses to root temperature were observed during plant aging.

To measure biomass, root morphology and C as well as N concentrations of plants grown at different root temperatures, the plant pots (Chap.2.3.1) were positioned in the root temperature boxes. There, plants germinated and grew at uniform root temperatures of 10°C, 15°C and 20°C RT as well as at 20-10°C RTG. The development stage of plants was determined three times a week. According to BBCH guidelines (Meier, 2001), development stages are defined as the following (Fig. 8):

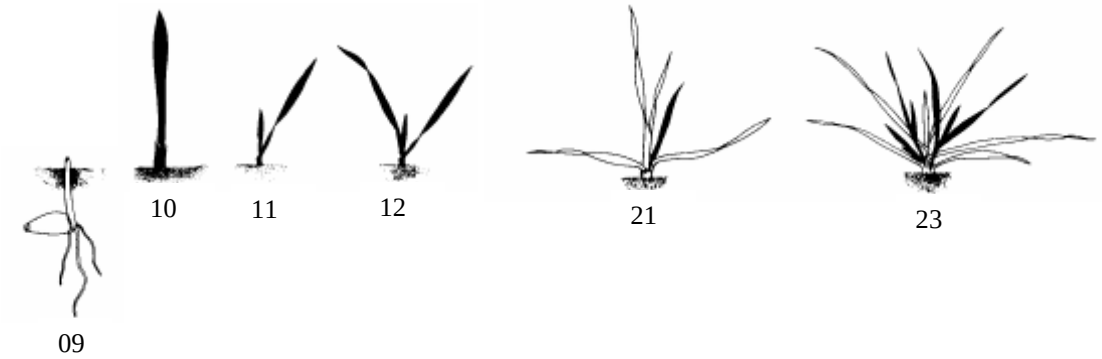

Fig. 8: Developments stages of barley as defined by Meier (2001); ©1989, Bayer.

(1) The barley plants were harvested 30 days after germination for comparing the influence of root temperature on plants of same age. (2) Additionally, plants were grown at 20°C RT until the first tiller developed (after 24 days). This developmental stage was the same one plants reached after 30 days when grown at 10°C RT. (3) Plants grown at 10°C and 20°C RT were harvested in a time sequence to examine changes in temperature effects due to plant aging. For that purpose, two plants at a time were harvested every forth day. Biomass (root and shoot separately), root morphology and relative growth rate were determined.
Barley grown at 10°C and 20°C RT was chosen for comparing plants at the same development stage, because these temperatures described the biggest differences between temperatures used in this study.

2.3.3 Data analyses

Biomass

At first, shoot and root were separated by a scalpel. Then, the plant pots were carefully divided into four horizontal layers (Fig. 7) Roots of each layer were washed out with deionized water and dried with paper tissue. Shoot and root fresh weight was determined immediately after harvest. Root weight was measured for each layer separately. Furthermore, fresh weight of the second developed leaf was determined. Afterwards, this leaf and about 100 mg root mass out of the first two layers (0-5 cm and 5-10 cm depth) were frozen in liquid nitrogen and stored in a -80°C freezer for further analyses. After drying the remaining plant material in an oven at 75°C until weight constancy, dry weights of all plant parts were determined. Dry weight of frozen plant parts was calculated by determining fresh weight (FW)/dry weight (DW) factor of the specific plant parts according to Eqn. 3 and then using Eqn. 4. In this way, it was possible to acquire total root and shoot dry weight including the parts which had been cut off before.

$$D = \frac{DW}{FW_{total} - FW} * 100 \qquad \textbf{Eqn. 3}$$

$$DW_{cut} = \frac{D}{100} * FW_{cut} \qquad \textbf{Eqn. 4}$$

D = dry weight factor

DW = measured dry weight

FW_{total} = fresh weight (before cutting off plant parts)

FW = fresh weight (without cut off plant parts)

FW_{cut} = fresh weight of cut off plant parts (either leaf or roots)

DW_{cut} = dry weight of cut off plant parts

Total plant dry weight, total root dry weight and root/shoot ratio were calculated.

Relative growth rate (RGR) was calculated out of dry weight data and shown as [%] (Eqn. 5):

$$RGR = \frac{\ln(X_2 / X_1)}{t_2 - t_1} * 100 \qquad \textbf{Eqn. 5}$$

X_1, X_2 = dry weight at different times

t_1, t_2 = different times

Leaf area

Leaf area of the second developed leaf was monitored by cutting off the leaf from the shoot and by outlining the leaf area on a blank sheet of paper. This paper leaf was cut out and as it was the substitute of the real leaf, the leaf area of the second developed leaf could be determined by measuring the weight of the paper leaf and calculating the leaf area with Eqn. 6:

$$LA = \frac{PW}{PD} \qquad \textbf{Eqn. 6}$$

PD = density of a blank sheet of paper [g cm^{-2}]

PW = weight of paper leaf [g]

LA = leaf area [cm^2]

Specific leaf area [cm^2 g^{-1} plant weight] was calculated by dividing leaf area by leaf weight (dry as well as fresh weight).

Root morphology

After harvest, root morphology of fresh roots was analyzed by a special scanner system and WinRhizo software (Régent Instruments Inc.), which is an image analysis system specifically designed for washed root measurements. Two light sources (one below the scanner glass and one in the scanner cover) allowed optimal illumination of the samples. Root length, surface area, and volume were measured with "REGENT's unique method". With this method measurements were made continuously at each point along the root. Overlap of rootlets, forks and tips were taken into account to provide accurate measurements of length and area. Additionally, the WinRhizo software checked root lengths measurements with an indirect statistical method based on Tennant (1975). This method determines the length of the spread root by counting the number of root intersections with vertical and horizontal lines of an underlying grid. Root length and number of intersections are related.

Root tissue density [$g\ cm^{-3}$] was calculated by dividing root dry weight by root volume.

Root diameter differences could also be analyzed by WinRhizo software. However, the frequency of different root diameters occurring for barley had to be analyzed before using the data delivered by the WinRhizo software. A suitable diameter classification should be developed, which mirrored root diameter differences between the temperature treatments. Finally, root diameters were aggregated in classes of 0-1 mm, 1-2 mm and 2-3 mm with an error of ± 3 %. This error based on ground truth measurements obtained for wires of known diameter.

Carbon & nitrogen concentration

Carbon and nitrogen concentrations of shoots and roots in the different substrate layers were analyzed at Central Division of Analytical Chemistry (Research Centre Juelich). The dry plant material (either shoot or root) of each plant was individually and finely grounded in a pebble mill or, if there was very little plant material left, by hand in an agate mortar and analyzed by CHNS-Analyzer (Leco CHNS-932).

2.4 Measurement of root temperature effects on root proliferation

2.4.1 Growth system

As it was not possible to follow root proliferation during experiments in plant pots, rhizotrones were used to measure this aspect. One side of these root boxes was covered with Perspex the other side was out of PVC. The rooting space was about 533 cm^3 (height: 26.0 cm, width: 20.5 cm, depth: 1.0 cm). Rhizotrones were positioned at an angle of ~ 45° with Perspex side faced to the floor and covered with aluminum foil to keep roots in dark (Fig. 9). Roots grew along the Perspex and could easily been followed. Pulse irrigation (30 ml once a day) was sufficient to maintain similar conditions as in plant pots due to the same preparation steps (cf. Chap. 2.3.1). Seed position and nutrient solution changes were analogous to the plant pot system (cf. Chap. 2.3.1).

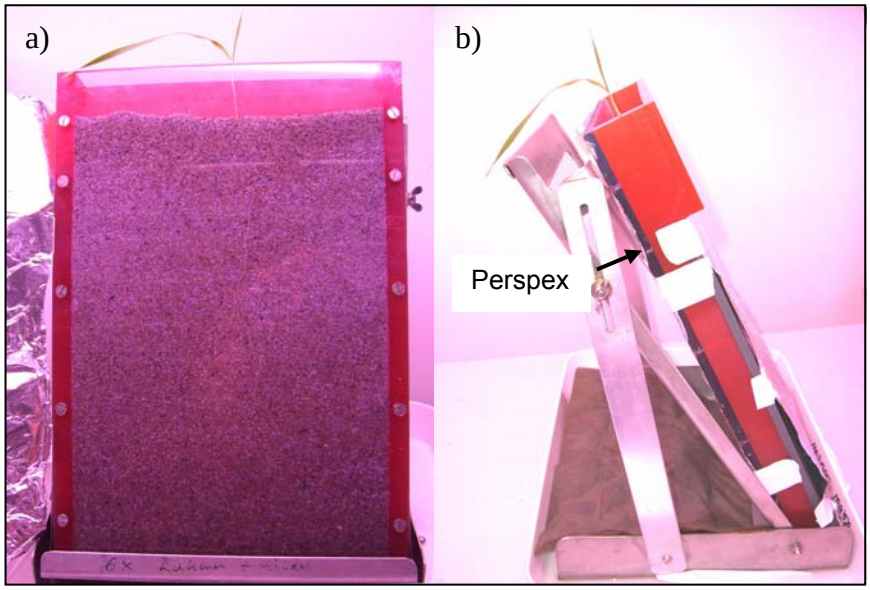

Fig. 9: Rhizotrone with barley: a) front sight; b) at an angle of about 45°.

2.4.2 Experimental protocol

Plants were germinated in rhizotrones at room temperature (22 ± 1°C), since all plants should be of same age and size at the beginning of the experiment. Rhizotrons were inserted in the temperature boxes two days after barley germination. Three plants were grown per treatment according to root temperatures listed in Tab. 1. Temporal changes in root temperature were chosen to figure out differences in effects due to the direction of temperature change. Effects

caused by a vertical root temperature gradient could not be observed due to technical reasons. Development stage (Fig. 8), number of seminal and first order lateral roots as well as root elongation of the seminal roots was monitored three times a week. The growth rate of seminal roots was measured manually. The number of seminal and first order lateral roots was counted by hand. At harvest time (30 days after germination) biomass, root morphology and number of seminal as well as first and second order lateral roots was determined characterizing responses of root architecture (e.g. branching) to root temperature. Analyses of biomass and root morphology were analogous to those described in Chap. 2.3.3. However, here roots were analyzed in total.

	Treatment			
week	**10°C**	**A**	**B**	**20°C**
1	10°C	10°C	20°C	20°C
2		15°C	15°C	
3				
4		20°C	10°C	

Tab. 1: Rhizotrone experiments used for determining root architecture. Scheme of root temperature treatments and their temporal changes are shown.

2.5 Measurement of root temperature effects on nutrient uptake kinetics of barley

2.5.1 Growth system

A hydroponical growth system was used to measure dynamic processes inside the plants. Barley seedlings germinated and grown in vermiculite for 2-3 days were transferred to hydroponics. The hydroponic system was build from black, aerated plant pots (volume: 1 L) covered with a lid to avoid algae growth (Fig. 10). Again, the solution switched from tab water to 0.5 modified Hoagland solution 9 days after germination (cf. Chap 2.3.1). The solution was renewed once a week to avoid nutrient depletion.

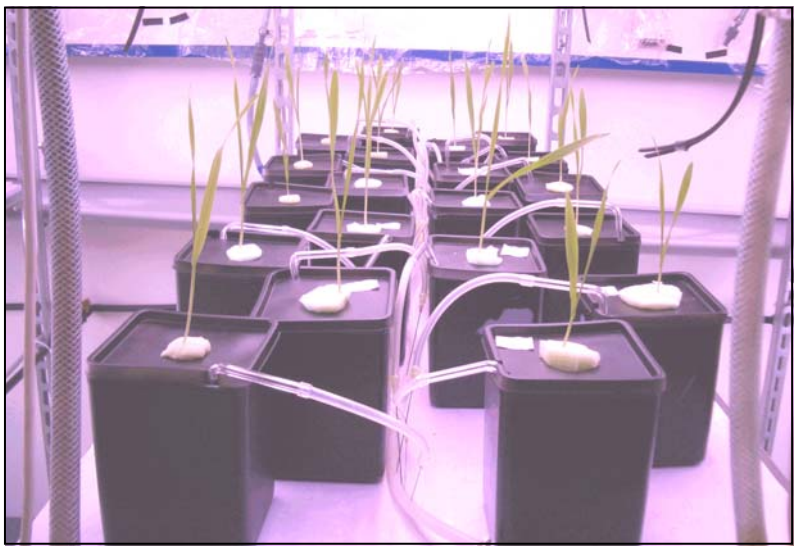

Fig. 10: Hydroponic system. Plants were pre-grown in this system before they were positioned in the experimental setup.

2.5.2 Experimental design

Temperature effects on nutrient uptake and transport within the plant were analyzed by an experiment on *nutrient uptake kinetics*. Plants grew in hydroponics at room temperature until they reached development stage 12-13 (3-4 leaf stage). They did not differ in biomass or root morphology. One day prior to the kinetic experiment, plants were transferred to beakers containing 150 ml aerated nutrient solution. They were inserted into the temperature boxes to adapt to new surrounding conditions of 10°C, 15°C and 20°C RT, respectively. The vertical root temperature gradient treatment was excluded, because it was not possible to generate a temperature gradient in this setup. The next day, nutrient solution was exchanged with 150 ml temperature adapted nutrient solution of chemically identical composition, but labeled with stable isotopes. The stable isotopes ^{15}N, ^{25}Mg and ^{44}Ca were chosen and provided as $NaNO_3$ (98 % ^{15}N), $MgSO_4$ (97.2 % ^{25}Mg) and $Ca(NO_3)_2$ (50 % enriched with 97 % ^{44}Ca only at the 24 h interval) in Hoagland solution. In this way, an active and a passive uptake system could be observed (e.g. Glass *et al.*, 1992; Kuhn *et al.*, 2000; Ferguson & Clarkson, 1976).Three plants of each root temperature treatment were sampled at a time either after 15 min, 30 min, 1 h, 3 h, 6 h, 9 h, 24 h or 48 h of labeling. The labeling solution of the 48 hours treatment was renewed after 24 hours.

2.5.3 Data analyses

Biomass

At harvest, labeled plants were taken out of the hydroponics and were shortly shaken in unlabeled 0.5 Hoagland solution to remove most of the adsorbed tracer elements. Then, roots were immediately dried with paper tissue and shoot and root were separated by a scalpel. The further biomass determination followed the protocol described in Chap. 2.3.3.

Nutrient uptake and translocation

Analyses were performed at shoot material of each individual plant, while root data were gathered from pooled material of the three plants harvested at a time.

Chemical analysis of ^{15}N was conducted at the Institute of Crop Science and Resource Conservation, Department of Plant Nutrition (Rheinische Friedrich-Wilhelms-University Bonn). Dried plant material was finely grounded in a so called “Trabbi mill” (a pebble mill driven by a Trabbi-motor, University Bonn, self-made) or, if there was less than 100 mg dry mass, by hand in an agate mortar. To determine isotope ratios and N concentrations, 5-6 mg plant material per sample were weighed into tin capsules and measured by an automatic nitrogen and carbon analyzer with integrated gas chromatograph coupled to a mass spectrometer (ANCA-SL/2020; Europa Scientific (SerCon), Crewe, Cheshire, UK). Calibration occurred by wheat flour with 1.61 % N and 0.3674 atom % ^{15}N.

Measurement of ^{25}Mg and ^{44}Ca was conducted by the Central Division of Analytical Chemistry (Research Centre Juelich). Dried and grounded plant material as mixture with HNO_3, H_2O_2 and HF was disintegrated by microwave. Then, diluted sample solution was analyzed by ICP-MS (Inductively Coupled Plasma with Mass Spectroscopy, Elan 6000, Perkin Elmer, Sciex; Agilent 7500ce, Planitz). Total Mg concentration was acquired by the signal of the sum of all Mg isotopes (^{24}Mg, ^{25}Mg, ^{26}Mg) separately determined before. Total Ca concentration was analyzed by ICP-MS and isotopes were determined by a newly developed analytical method using quadrupole-based ICP-MS with octopole collision cell (personal communication Becker & Seeling; paper submitted).

The labeled fraction on the respective nutrient (^{15}N/total N, ^{25}Mg/total Mg and ^{44}Ca/total Ca), total content of the stable isotope as well as its distribution between root and shoot were calculated for the different time intervals.

2.6 Determining the influence of root temperature & plant structure on nutrient uptake

The *nutrient uptake in sand* experiment was conducted on the one hand to check, whether temperature effects occurring in the *nutrient uptake kinetics* experiment (cf. Chap. 2.5) also revealed in the natural conditions predominating in the other experimental setups; and to examine possible temperature effects on nutrient uptake caused by the 20-10°C RTG treatment. On the other hand it was analyzed, whether adaptation of plant structure to the respective root temperatures caused different results for nutrient uptake than seen for plants only exposed to experimental root temperatures for labeling.

2.6.1 Experimental design

Plants were grown in plant pots prepared as described in Chap. 2.3.1 at room temperature until they were 29-30 days old. Consequently, all plants reached the same development stage (here stage 13 = 4 leaf stage), similar total biomass, root and shoot masses and especially root morphology. At day 29-30 plants were positioned in the temperature boxes at 10°C, 15°C, 20°C RT and 20-10°C RTG. The adaptation of substrate temperature to experimental temperatures lasted three hours. Immediately after inserting plants, 50 ml of Hoagland solution containing enriched $NaNO_3$ (98 % ^{15}N) and $MgSO_4$ (94.5 % ^{25}Mg) were added by syringe (Sterican® 0.90 x 70 mm, 20 G x 2¾″, Braun) in 5 ml pulses. Pulses were injected from the top of the plant pot either in 1 cm (treatment A) or in 6 cm (treatment B) depth below the sand surface. They were equally distributed around the shoot. This allowed an even distribution of labeled nutrient solution within the plant pots (extensively tested in a preliminary experiment with dye (Brilliant Blue); data not shown); and ensured that all root parts within one depth were able to absorb tracers. Labeling in two depths should give more details about the differences in uptake due to root type and root structure involved in this process: Labeling in 1 cm depth represented uptake by the entire root system, whereas labeling in 6 cm depth implied uptake only by part of the root system. Labeling was repeated three times in three hour intervals (150 ml labeling solution in total; corresponding to the amount of nutrient solution in the *nutrient uptake kinetics* experiment, cf. Chap. 2.5.2). Plants were harvested 9 hours after the first labeling.

The *nutrient uptake in sand* experiment was repeated with plants germinated and grown at the experimental temperatures. These plants were particularly adapted to the different root

temperature treatments in biomass and root structure. Therefore, it was possible to examine nutrient uptake affected by root temperature and structure together and to define differences between these results and results only caused by different root temperatures.

2.6.2 Data analyses

Biomass

In case of labeling experiments, analysis did not allow rinsing with deionized water, because ion exchange between plant material and the surrounding solution should be avoided. Therefore, after cutting off the shoot from root, roots were separated from sand by tweezers, were shortly shaken in unlabeled 0.5 Hoagland solution to remove most of adsorbed tracer elements and dried with paper tissue immediately. However, separating by tweezers was not as efficient as rinsing with water. Thus, some sand remained on the roots and a so called "sand factor" of ~ 20 % was calculated and taken into account when acquiring root dry weight in 0-5 cm and 5-10 cm depth of plants grown at 20°C RT and 20-10°C RTG. Here, the root system was extremely dense and it was very difficult to remove the sand completely.

Nutrient uptake and translocation

Analyses of the different stable isotopes followed the same protocol as described in Chap. 2.5.3. However, here all measurements were separately conducted for each individual plant and plant part. Root samples were mostly taken out of 0–5 cm and 5–10 cm depth, because of material limitations within the other depths.
The fraction of tracer (^{15}N and ^{25}Mg) on total N and Mg, respectively, ^{15}N and ^{25}Mg content as well as total N and Mg concentration in biomass were calculated.

2.7 Measuring compounds of the nitrogen metabolism

Nitrogen metabolism in plants grown at 15°C RT and 20-10°C RTG was observed more in detail, due to its importance in plant life. It should be analyzed, whether N metabolism of plants behaved in the same way at 20-10°C RTG and 15°C RT since 15°C represented the average temperature of the gradient. Frozen fresh plant material (second developed leaf and ~ 100 mg of roots in 0-5 cm and 5-10 cm depth) was finely grounded in liquid nitrogen in a

mortar and analyzed for nitrate, free amino acid concentration as well as for the concentrations of single free amino acids and for total proteins.

Nitrate

To analyze nitrate, plant material was homogenized in 1 ml bi-deionized water (MilliQ). Homogenates were centrifuged at 14,000 U min^{-1} and 4°C for 15 min. The supernatants were decanted and centrifuged again for another 15 min to remove final very fine plant residue. Aliquots were mixed with SA-H_2SO_4 (5% salicylic acid, w/v) and NaOH (2N) as described by Cataldo et al. (1975). Then, NO_3-N concentration was photometrically measured at 410 nm wave length.

Free amino acids

The concentration of different free amino acids in plant material was determined by homogenizing samples in 500 µl extracting agent (ethanol and 0.1 M HCl v/v 1:1) and 20 µl internal standard (1 mM ACH) within 3 min. Other 500 µl extracting agent was used to remove all plant extract out of the mortar. The homogenates were centrifuged at 14,000 U min^{-1} and 4°C for 15 min. Then supernatants were removed and used for free amino acid measurements. The measurements were conducted with a method developed by the BioSpec working group (Research Centre Juelich, Stein & Oldiges, personal communication) using LC-MS (Liquid Chromatography coupled with Mass Spectroscopy) with Phenomenex Luna 5µ SCX 100A (150 x 2 mm) column. Additionally, ^{15}N labeled samples were analyzed. In total, $^{15}N/^{14}N$ ratio of 13 free amino acids could be determined.

Proteins

The total amount of proteins in fresh plant material was determined according to Bradford (1976). To extract proteins, plant material was homogenized and diluted with buffer solution (HEPES). After mixture with dye reagent, the total solution was photometrically analyzed at 595 nm wave length and protein concentration was calculated.

2.8 Statistics

All statistical analyses were performed by SigmaStat 2.03 software and diagrams were plotted by SigmaPlot 2001 software (both SPSS Inc.). Statistical methods were t-test, one way

Analysis of Variance (ANOVA) or Kruskal-Wallis one way ANOVA on ranks, if normality test failed. Kruskal-Wallis one way ANOVA on ranks determines statistically significant differences between medians. Therefore, t-test was additionally performed describing statistically significant differences between means as favored in this study. Pair wise multiple comparison procedures at ANOVA were done by Tukey Test or Dunn's Test if two different sample sizes were compared and normality test failed.

3 Results

3.1 Plant development & plant growth

3.1.1 Germination

The first time, when plants experience temperature impact in their life cycle is during germination. In this study, germination was defined as the stage when the coleoptile emerged at the surface (development stage 09; Meier, 2001; Fig. 8). Barley seeds needed nearly twice the time to germinate at 10°C uniform substrate temperature compared to seeds at 20°C (9.2 and 5.1 days, respectively). A slight delay of germination occurred at 15°C substrate temperature (6.3 days; Fig. 11). As expected, no differences between times until germination occurred, when seeds germinated at 20°C and at 20-10°C vertical gradient in substrate temperature.

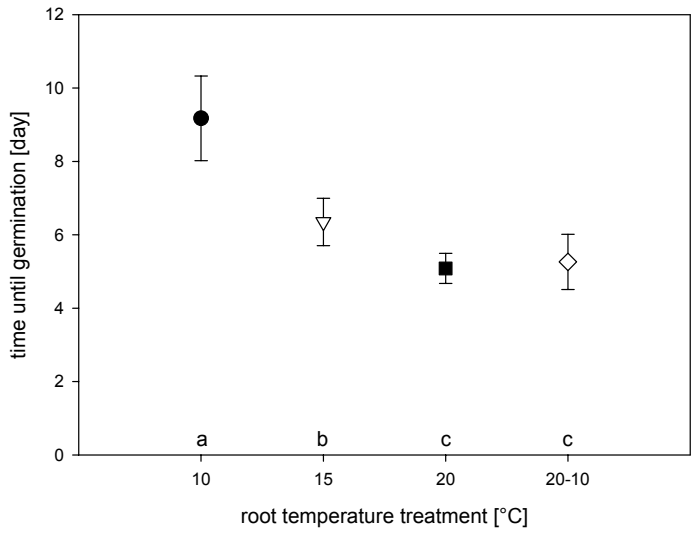

Fig. 11: Time until germination of barley seeds in days. Mean and standard deviation are shown. Statistical analysis was performed by one way ANOVA; $p < 0.05$; $n = 24$; different letters mark statistically significant differences.

3.1.2 Plant development stages

To figure out, whether root temperature controls development speed of aboveground plant parts, development was monitored and characterized as described by Meier (2001; Fig. 8). Results showed that three different periods had to be distinguished (Fig. 12):

(1) The aboveground development speed of plants was the same at all temperature treatments until day 10 after germination. At this time, the first leaf was developed and the tip of the

second leaf was visible (stage 11) at plants of all temperature treatments. (2) During the following period (day 10-25) development accelerated, especially when plants were grown at the vertical root temperature gradient. Development of plants grown at 15°C and 20°C uniform root temperature (RT) occurred nearly simultaneously, but slightly slower than development of plants grown at the 20-10°C vertical gradient in root temperature (RTG). In contrast, the development of plants grown at 10°C RT was delayed. (3) Exceeding day 25, development speed of plants slowed down, except when roots were exposed to the vertical root temperature gradient even though they were already physiologically older. Consequently, plants grown at the different root temperature treatments had reached different development stages at harvest time (30 days after germination). At this time, plants grown at 15°C and 20°C RT showed mean development stages of 22.7 and 23.7 (2.7 and 3.7 tillers developed, respectively), whereas plants grown at 10°C RT only reached a mean of 20.7 (0.7 tillers). Plants grown at vertical root temperature gradient developed most tillers within 30 days (stage 27 = 7 tillers).

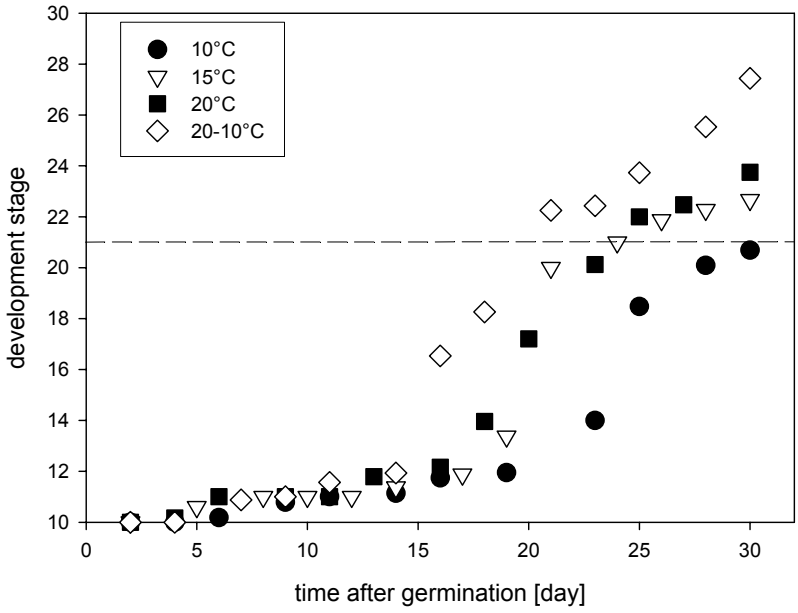

Fig. 12: Time series of aboveground plant development after germination at different root temperature treatments. Development stages indicate number of leaves (stage 10-20) and shoots (stage 21-29). For example: stage 11 = one developed leaf and at least tip of the second leaf visible (cf. Fig. 8); dashed line =stage 21, main stem and first tiller visible. Mean is shown, n = 24.

3.1.3 Leaf structure

A closer look on aboveground biomass development demonstrated that plants grown at the vertical root temperature gradient did not only reach the highest development stage, but also produced a disproportionate amount of leaves. This resulted in a higher leaves/shoot ratio than obtained at the other root temperature treatments (Fig. 13).

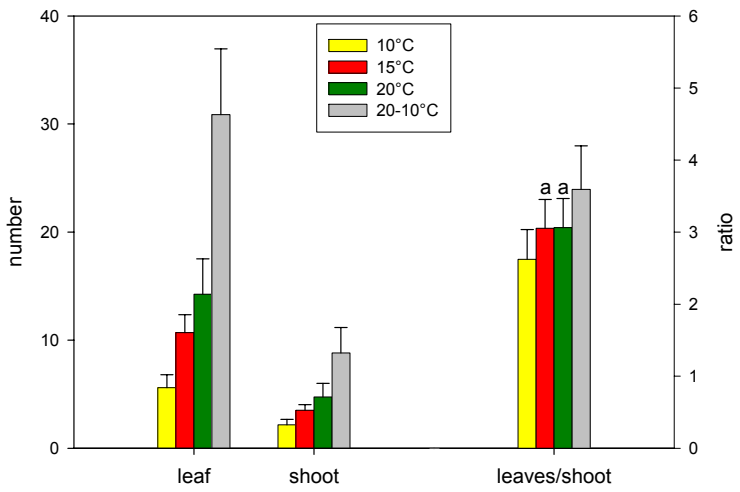

Fig. 13: Aboveground biomass – number of leaves, total shoots and ratio of leaves per shoot of plants grown at different root temperature treatments. Mean and standard deviation are shown. Statistical analysis was performed by one way ANOVA and t-test; $p < 0.05$; $n = 24$; a = not significantly different.

No significant difference in leaf area of the second developed leaf was observed between plants grown at uniform root temperatures and in the temperature gradient. However, plants grown at 20°C RT reached the largest leaf area of plants grown at uniform root temperatures (Fig. 14a). Although all plants showed a leaf area within the same range, the specific leaf area of plants was significantly smaller at 20-10°C RTG compared to uniform root temperatures (~ 440 cm^2 g^{-1} dry weight vs. ~ 500-540 cm^2 g^{-1} dry weight; Fig. 14b). Thus, the leaves produced at uniform root temperatures were less dense.

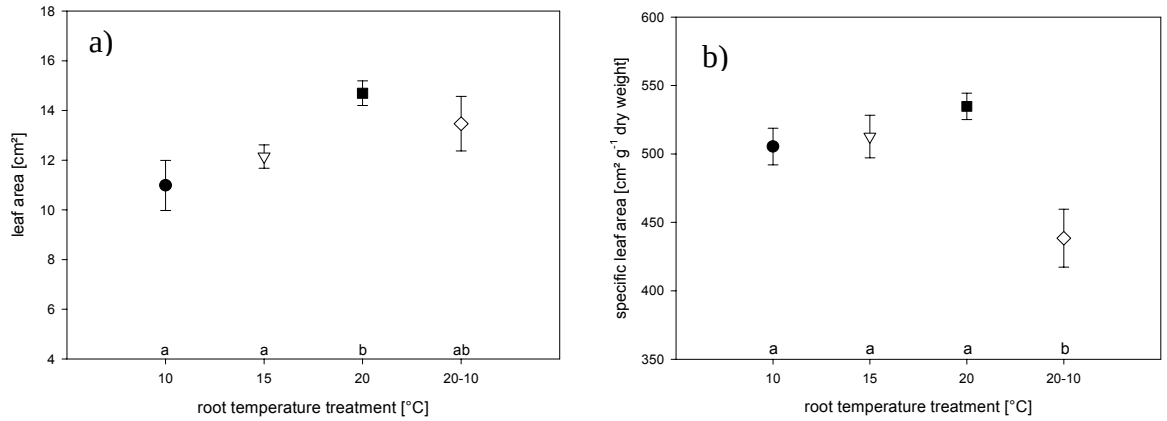

Fig. 14: a) Leaf area of the second developed leaf at different root temperature treatments. b) Specific leaf area in [cm²] leaf area per [g] dry weight of the second developed leaf. Mean and standard error of the mean are shown. Statistical analysis was performed by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences.

3.1.4 Biomass of plants compared at the same age

The impact of root temperature on total biomass as well as on shoot and root masses was analyzed. Biomass production was significantly enhanced by the vertical root temperature gradient as already seen with development speed of plants. Shoot (i.e. total aboveground plant parts) dry weight increased with increasing temperature in uniform root temperature treatments, but the highest value was reached when plants were grown at the vertical root temperature gradient (Fig. 15; App. 17). Similar positive correlations between increasing uniform root temperature and biomass gain were observed for root mass. However, no significant difference was observed between root dry weight of plants grown at 15°C and 20°C RT. Again plants grown at vertical root temperature gradient produced most biomass (Fig. 15; App. 17).

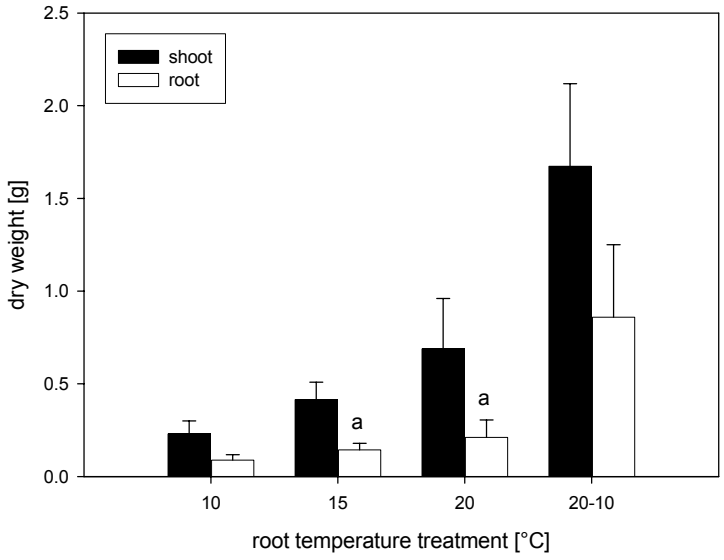

Fig. 15: Shoot and total root mass measured as dry weight [g] of plants grown at different temperature treatments. Shoot mass = total aboveground biomass. Mean and standard deviation are shown. Statistical analysis was performed by t-test. It was conducted within the individual temperature treatments as well as between the treatments ($p < 0.05$); a = no significant difference; n = 8.

Only weak differences occurred in root/shoot biomass ratio (Fig. 16), although the total amount of biomass remarkably varied between the temperature treatments. Nevertheless, a slight tendency towards higher root/shoot ratio was seen when plants grew at vertical root temperature gradient (Fig. 16: ratio of 0.5 vs. ~0.4; App. 17: ratio of ~1.0 vs. ~0.4).

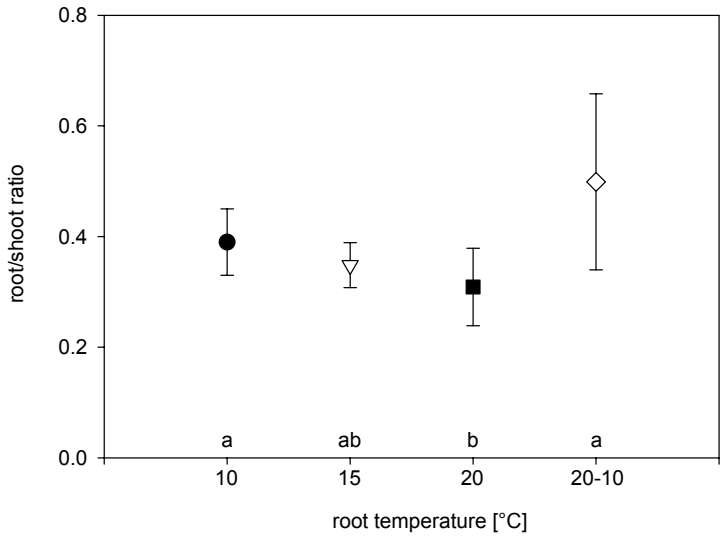

Fig. 16: Root/shoot biomass ratio of plants grown at different root temperature treatments. Mean and standard deviation are shown. Statistical analysis was performed by one way ANOVA and t-test; $p < 0.05$; n = 8. Different letters mark statistically significant differences.

3.1.5 Summary

Germination of barley seeds was delayed by decreasing soil temperatures. Higher root temperatures and especially the vertical root temperature gradient accelerated plant development and biomass production, whereas biomass allocation to root and shoot was

independent of root temperature. Nevertheless, root growth appeared to be stimulated by the vertical root temperature gradient, indicated by slightly shifting root/shoot biomass ratio closer to one. In addition, the vertical root temperature gradient caused morphological changes regarding leaf tissue density and leaf emergence per shoot.

3.2 Root morphology of plants compared at the same age

Root temperature might not only affect barley at the entire plant level. Especially the directly exposed roots could also be influenced in their growth and structural development. The root system was examined in detail to prove this hypothesis.

3.2.1 Root mass distribution with depth

The influence of root temperature on rooting depth and thus on vertical expansion of the possible water and nutrient exploitation volume was examined by analyzing root mass distribution with depth. Root distribution followed the same pattern at all temperature treatments: the fraction of total root dry weight decreased with rooting depth. In 0-5 cm depth the fraction was highest with 65-75 % of total root weight, whereas it decreased to 15-25 % and 5-15 % in 5-10 cm and 10-15 cm depth, respectively (App.1a,b). Only plants grown at 20°C RT explored 15-20 cm depth with 5 % of their root mass. The values reached in the other temperature treatments were negligible or no roots were found at all (15°C RT treatment).

Regarding the absolute root dry weight distribution with depth, differences became more distinct. Plants grown at 20°C RT mainly differed from plants grown at the other uniform root temperatures by higher amounts of dry weight in the deeper substrate layers (10-15 cm and 15-20 cm; Fig. 17). In contrast, plants grown at the vertical root temperature gradient showed remarkably high root dry weights within 0-5 cm and 5-10 cm depth (Fig. 17), whereas in 10-15 cm depth no significant difference occurred compared to plants grown at the 10°C and 15°C RT treatment. This was especially remarkable, because due to high total root mass of plants grown at 20-10°C RTG one might have expected increased rooting into depth, as seen for plants grown at 20°C RT. In conclusion, the higher total biomass of plants grown at the vertical gradient in root temperature was not supported by deeper roots, but by more roots in the upper layers.

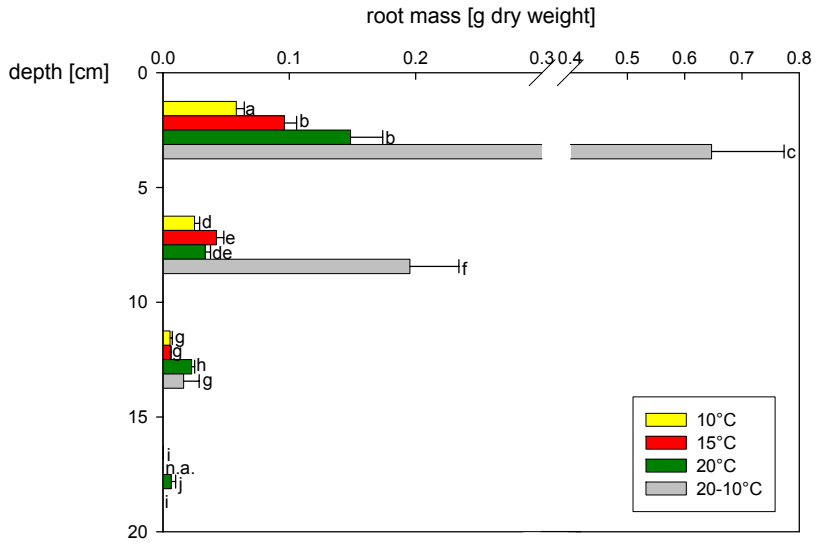

Fig. 17: Root mass [g dry weight] at different depths. Mean and standard error of the mean are shown. Statistical analysis was performed by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences; n.a. = not available.

3.2.2 Root surface area distribution with depth

Structural changes in root morphology might also be relevant with respect to nutrient and water uptake functions. Thus, root surface as potential uptake area was quantified in the treatments. Closely related to total root dry weight, total root surface area significantly differed between all root temperature treatments (Fig. 18). Highest absolute surface area was always reached in 0-5 cm depth (Fig. 19; 50-60 % of total root surface area, App. 2). It decreased with depth in uniform root temperature treatments. No significant difference in surface area was detected between 0-5 cm and 5-10 cm depth when plants were grown at 20-10°C RTG (App. 5), even though root dry weight was much higher in 0-5 cm depth (Fig. 17; App. 4).

Possible changes in nutrient uptake properties due to root temperature variation were indicated by regarding root surface area in context with total plant dry weight. The lower value at 20-10°C RTG compared to uniform toot temperature treatments (0.018 m^2 g^{-1} vs. ~ 0.032 m^2 g^{-1} dry weight; Fig. 20) indicated less surface area potentially available for supplying one unit plant biomass with nutrients and water.

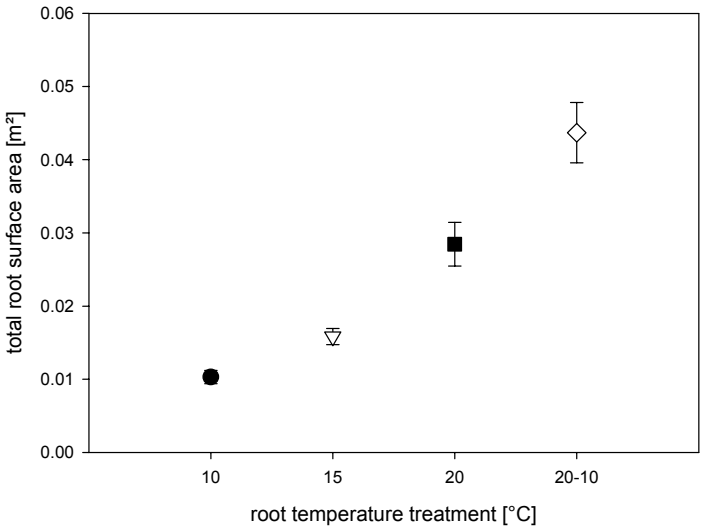

Fig. 18: Total root surface area of plants grown at different temperature treatments. Mean and standard error of the mean are shown. Statistical analysis was performed by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Statistically significant difference occurred between all treatments.

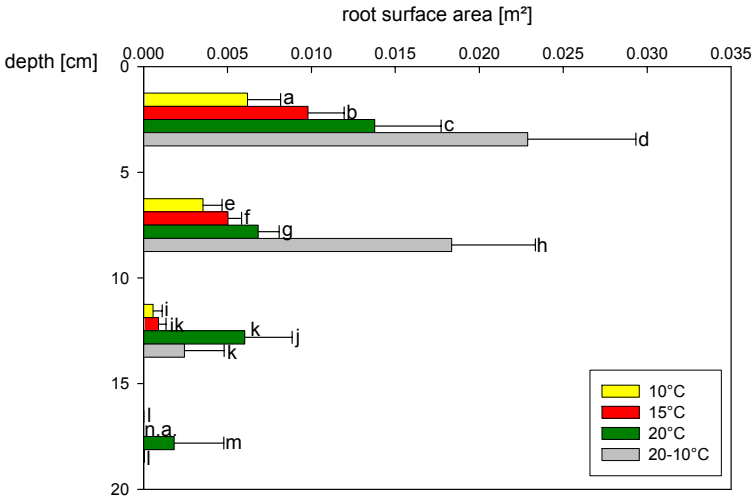

Fig. 19: Root surface area at different depths. Mean and standard error of the mean are shown. Statistical analysis was performed by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences; n.a. = not available.

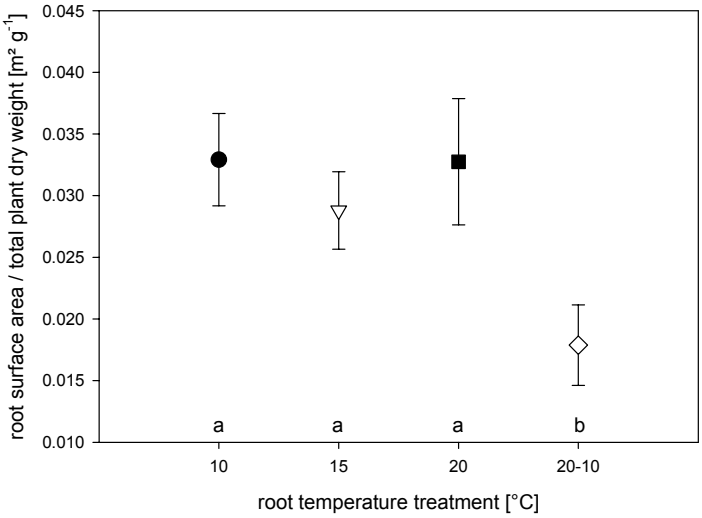

Fig. 20: Potential uptake area supplying a unit of plant biomass. Mean and standard deviation are shown. Statistical analysis was performed by one way ANOVA; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences.

3.2.3 Root length distribution with depth

Characterizing lateral and vertical expansion of the potential exploitation area of roots, root length is an additionally important variable.

Although total root dry weight of plants grown at the vertical root temperature gradient was much higher than of plants grown at 20°C RT (0.86 g and 0.21 g, respectively) total root length was the same (Fig. 21). This effect was also detected for plants grown at 10° and 15°C RT. Here, root dry weight of plants grown at 15°C RT was 38.45 % higher than of plants grown at 10°C RT, but total root length at both treatments ranged between 4.0 m and 6.0 m and showed no statistically significant difference (Fig. 21). Root length of plants grown at 20°C RT and 20-10°C RTG exceeded that of plants grown at 10°C and 15°C RT 3-4 times, although no significant differences in root dry weight occurred between plants grown at 15°C and 20°C RT (Fig. 15).

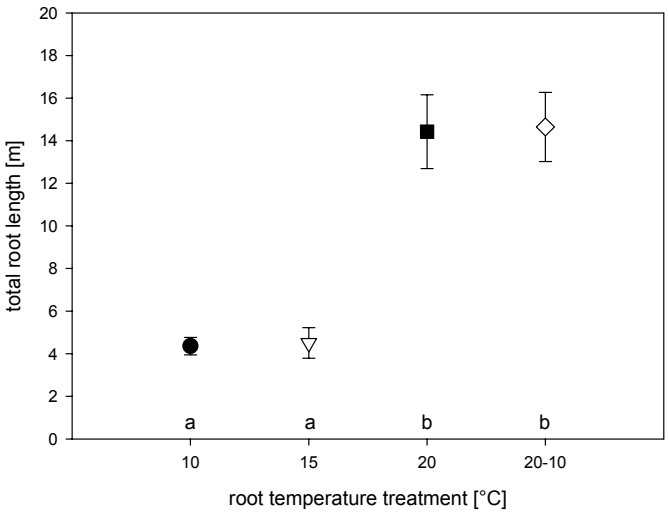

Fig. 21: Total root length of plants grown at different root temperature treatments. Mean and standard error of the mean are shown. Statistical analysis was performed by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences.

Root length fractions on total root length decreased with depth at all uniform root temperature treatments (App. 3), as this was already seen for the distribution of root dry weight fraction (Chap. 3.2.1). In contrast, fraction of total root length of plants grown at 20-10°C RTG was highest in 5-10 cm depth (App. 3). Corresponding to the root length fractions, absolute root length at all uniform temperature treatments was highest in 0-5 cm depth, while plants grown at 20-10°C RTG reached the highest value in 5-10 cm depth (Fig. 22). However, this root length did not significantly differ from root length in 0-5 cm depth at 20-10°C RTG (Fig. 22; App. 6). The decrease in absolute root length with depth was lower at 20°C RT compared to the other uniform temperatures due to a more even distribution of root length which did not

significantly differ between the three uppermost substrate layers (0-15 cm depth, Fig. 22; App. 6).

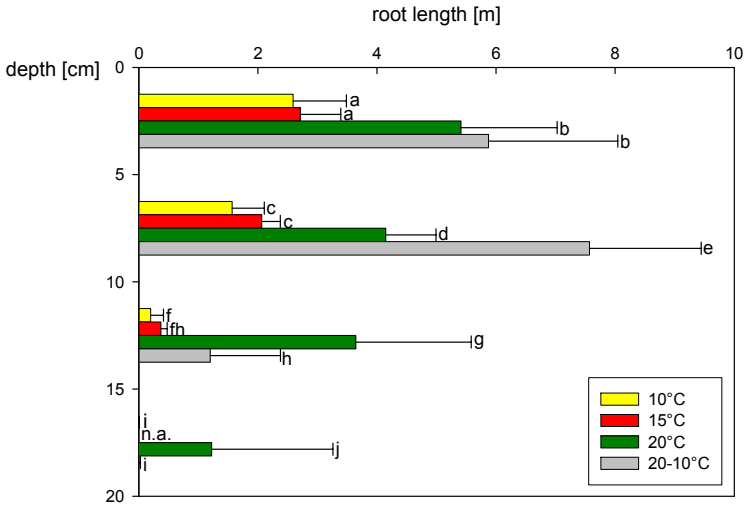

Fig. 22: Root length at different depths. Mean and standard deviation are shown. Statistical analysis was performed by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences; n.a. = not available.

Despite differing root dry weights (0.058 g and 0.096 g, respectively; Fig. 17), plants grown at 10°C and 15°C RT reached the same root length within 0-5 cm depth (2.56 m and 2.71 m, respectively; Fig. 22). The same effect was obtained comparing plants grown at 20°C RT and 20-10°C RTG. However, roots within 0-5 cm depth were twice as long at 20°C RT and 20-10°C RTG compared to 10°C and 15°C RT (Fig. 22). Disproportion between root dry weight and root length was also observed within a single temperature treatment (App. 4 and App. 6): e.g. roots of plants grown at 20°C RT did not significantly differ in root length in 0-5 cm and 5-10 cm depth; although root dry weight was in mean 77.46 % lower in 5-10 cm depth (Fig. 17). These results in addition to observations made for root surface area and root dry weight (Chap. 3.2.1 and Chap. 3.2.2) indicated changing root morphology due to soil temperature especially in 5-10 cm depth. It was hypothesized, that either roots in 5-10 cm depth had to be thinner and/or lighter than in 0-5 cm depth when plants were grown at 20°C RT and 20-10°C RTG.

3.2.4 Root tissue density & root diameter

Hardly any significant change in root tissue density – defined as root dry weight [g] to root volume [cm^3] - between root temperature treatments and between root depths was detected (App. 7). This supported the hypothesis that root temperature causes stronger variation in root diameter than in root tissue density. At entire root level fractions of different root diameters on total root length seemed to be independent of absolute total root length, but they differed

between the root temperature treatments. Thin roots (0.0-1.0 mm diameter) dominated with fractions of 75 % to 85 % (Fig. 23). This fraction was highest, when plants were grown at 20°C RT compared to any other temperature treatment and was associated with smallest fractions of thicker roots (> 1.0 mm diameter). Remarkably, root diameter fractions of plants grown at 15°C RT and 20-10°C RTG were exactly the same.

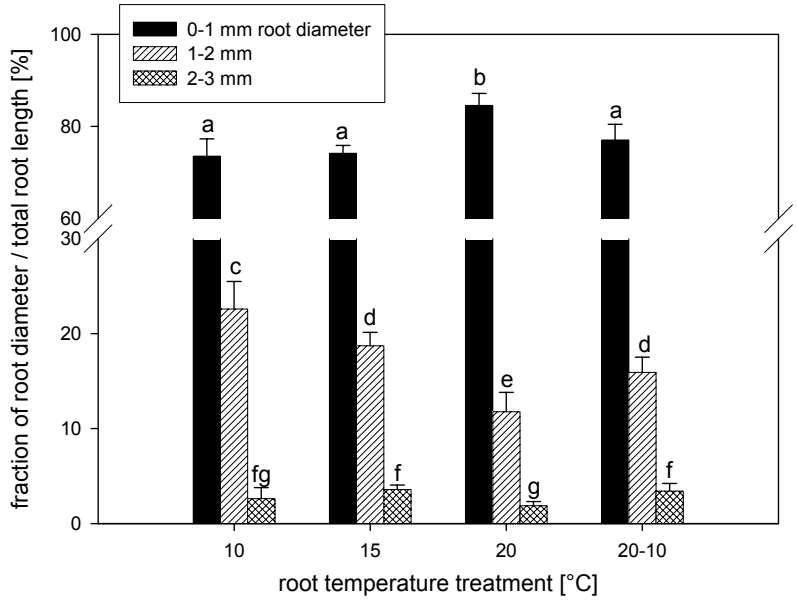

Fig. 23: Fraction of different root diameters on total root length. Mean and standard deviation are shown. Statistical analysis was performed by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences.

The distribution of the above mentioned fractions of individual root diameters was determined with depth (Fig. 24). Thin roots (0.0-1.0 mm diameter) dominated in each depth independent of root temperature treatment. Furthermore, the main fraction of roots with diameters > 2.0 mm appeared in 0-5 cm depth. Nearly no thicker roots (> 2.0 mm diameter) were present in 5-10 cm depth, except for plants grown at vertical root temperature gradient and to less extend at 15°C RT. They were absent in deeper substrate layers. The pattern of diameter distribution with depth was quite similar between plants grown at 10°C and 15°C RT. At both temperatures, fraction of roots with 0.0-1.0 mm diameter was 40 % and 30 % in 0-5 cm and 5-10 cm depth, respectively. Again, plants grown at 20°C RT showed more uniform data (Chap. 3.2.3). Here, fraction of 0.0 - 1.0 mm diameter roots was nearly 30 % in each layer, except in 15-20 cm depth (Fig. 24).

Although total root diameter fractions of plants grown at 20-10°C RTG were the same as of plants grown at 15°C RT, their distribution with depth differed. At 20-10°C RTG, the root diameter composition (i.e. fractions of individual root diameters) in 0-5 cm depth was similar to roots at 20°C RT within the same depth. For this reason, it can be expected that root diameter composition in 5-10 cm depth corresponded with that at 15°C within the same depth,

due to comparable root temperatures between plants grown at 20-10°C RTG and 15°C RT within 5-10 cm depth. Remarkably, root diameter composition in 5-10 cm depth at 20-10°C RTG did not correspond with 15°C RT within the same depth, but was similar to that in 0-5 cm depth (Fig. 24).

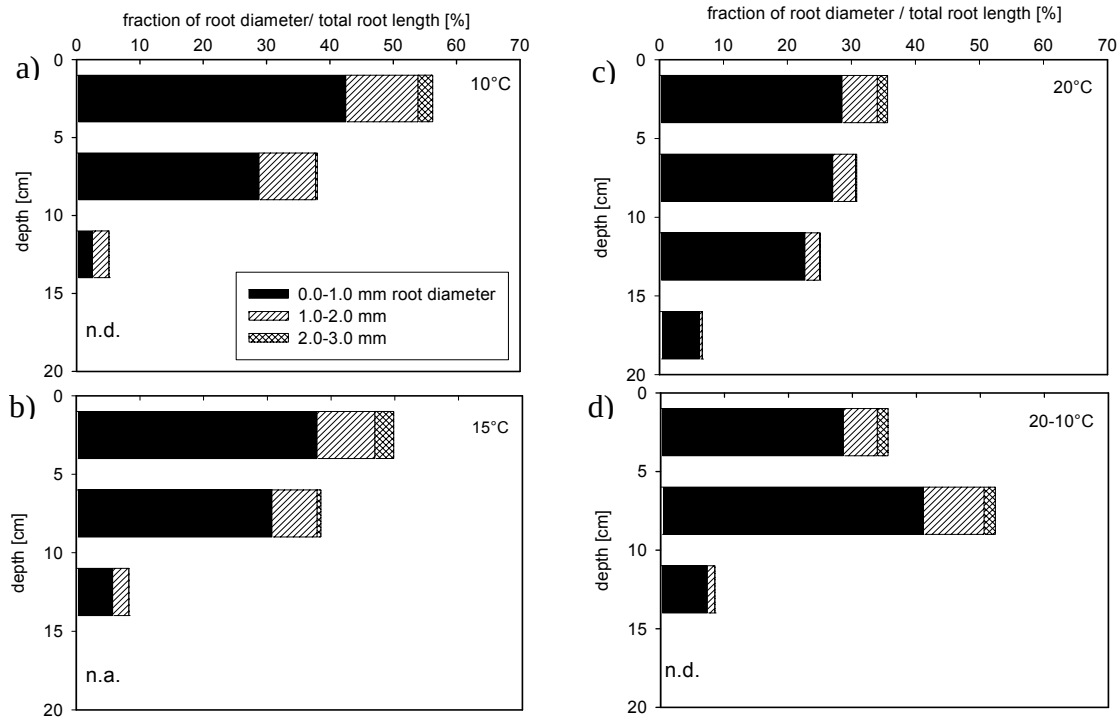

Fig. 24: Fraction of different root diameters on total root length at different depths. Plants were grown at a) 10°C, b) 15°C, c) 20°C uniform root temperature and d) 20-10°C vertical gradient in root temperature. Stacked bars show mean of each diameter class; n = 8; n.d. = not determined, n.a. = not available.

3.2.5 Root architecture

In this study, the influence of root temperature on root architecture (i.e. root branching) was determined by analyzing the number of lateral roots of different orders. Differences in total number of lateral roots (here: 1st and 2nd order laterals) were identified in plants grown at two constant (10°C; 20°C) and at two temporally changing root temperature treatments (temporal change: 1 week – 2 weeks – 1 week; temperature treatment A: 10°C to 15°C to 20°C and temperature treatment B: 20°C to 15°C to 10°C) in rhizotrones for 30 days (cf. Chap. 2.4.1). The number of 1st order laterals did not significantly differ between the temperature treatments, whereas 2nd order laterals significantly varied between the temporally changing temperature treatments (Tab. 2). Plants in treatment A produced significantly less 2nd order laterals (mean 119 ± 19.03 %) than plants grown at treatment B (mean 156.67 ± 2.24 %;

Tab. 2). In contrast to plants grown at the other temperature treatments, plants grown at treatment B seemed to invest more in lateral root production than into seminal roots (Fig. 25).

Root temperature treatment	Dry weight [g]		Root length [cm]	Root surface [cm²]	Number of lateral roots at harvest (day 30)		
	Shoot	Root			1st order	2nd order	Sum
10°C	0.04	0.05	172.40[a]	56.64[a]	105	120[ab]	225[ab]
A	0.04	0.04	235.24[ab]	60.84[ab]	80	119[a]	199[a]
B	0.06	0.07	429.10[c]	122.09[b]	87	156[b]	244[b]
20°C	0.08	0.06	369.69[bc]	90.40[ab]	82	152[ab]	234[ab]

Tab. 2: Biomass, root architecture and root morphology of plants grown at different temperature treatments (temporally changing root temperature: A = 10-15-20°C; B = 20-15-10°C, cf. Tab. 1). Mean is reported. Statistical analysis was performed by one way ANOVA and t-test; $p < 0.05$; $n = 3$. Different letters mark statistically significant differences.

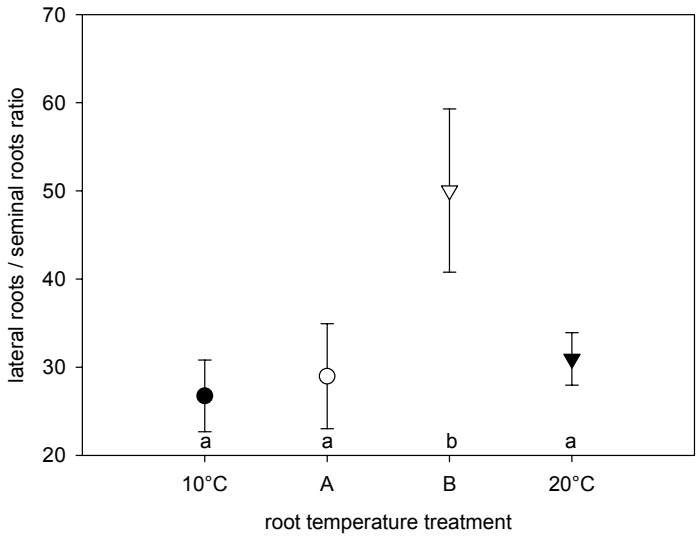

Fig. 25: Ratio of lateral roots to seminal roots at four different temperature treatments (temporally changing root temperature: A = 10-15-20°C; B = 20-15-10°C). Mean and standard deviation are shown. Statistical analysis was performed by t-test; $p < 0.05$; $n = 3$. Different letters mark statistically significant differences.

Furthermore, production of lateral roots increased by increasing plant age (Tab. 3). After two weeks of growth, the number of newly produced 1st order laterals was extremely increased at all temperature treatments, but differed in magnitude between them. Temporally changing root temperatures strongly influenced lateral root production. While the production of new laterals hardly differed between week three to four at constant root temperatures (10°C and

20°C), it strongly decreased at temporally changing root temperatures independent of the direction of change in temperature within this time (Tab. 3). Additionally, changing root temperature from 20°C to 15°C stimulated lateral root production, whereas no effect was caused by changing root temperature from 10°C to 15°C. Rather the latter one resulted in the same lateral root production as noticed at 10°C constant root temperature until reaching week 4. In conclusion, a temporal change from 20°C to 15°C root temperature stimulated lateral root production of younger plants and especially the production of second order laterals. In contrast, the dynamic of lateral root production was widely independent of the chosen constant root temperature conditions.

	week			
Root temperature treatment	**1**	**2**	**3**	**4**
	Newly produced 1st order lateral roots per week			
10°C	0.67	1.66	8.00	9.00
A	0.33	1.00	8.34	3.33
B	6.33	4.34	18.66	2.00
20°C	4.33	1.34	13.00	11.66

Tab. 3: Number of new 1st order lateral roots produced at a certain time at different root temperature treatments (temporally changing root temperature: A = 10-15-20°C; B = 20-15-10°C). Mean is reported; n = 3. Bars mark change in temperature at treatments A and B.

3.2.6 Root elongation

Beside root branching (cf. Chap. 3.2.5), elongation of individual roots is involved in formation of total root length and influences fractions of root diameters generating the total root length. Consequently, root temperature effect on root elongation rate was investigated. No significant differences were obtained in measuring the elongation rate of seminal roots. Therefore, the following results have to be interpreted as tendencies: Seminal roots showed less elongation per day at 10°C root temperature than at 20°C root temperature (Fig. 26). Furthermore, it was noticed, that root elongation rate of plants grown at temporally changing root temperatures adapted to the respective new root temperature. Hardly any difference was observed between elongation rate at 15°C and 20°C root temperature.

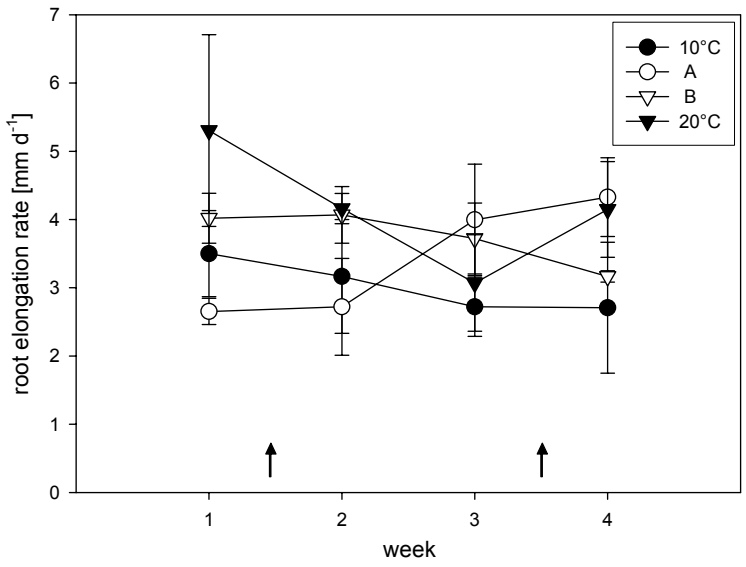

Fig. 26: Root elongation rate per day. Mean and standard error of the mean are shown. Statistically significant differences occurred neither between the root temperature treatments 10°C, A (10-15-20°C), B (20-15-10°C) and 20°C nor between the different weeks. Statistical analysis was performed by one way ANOVA and t-test; n = 3. Arrows mark temperature changes within treatments A and B.

3.2.7 Summary

Root dry weight distribution with depth depended on root temperature. It was more evenly distributed with depth at 20°C RT, while root dry weight of the other temperature treatments was mainly concentrated in the two uppermost substrate layers. Total root surface area correlated with total root dry weight, whereas its distribution with depth was more dependent on root diameter composition (i.e. fractions of different root diameters in entire root system). An even stronger correlation was observed between root diameter composition and root length distribution with depth. The variation in root diameter composition with depth enabled equal root lengths in different depths, although root masses differed. Remarkably, root length in 0-5 cm and 5-10 cm depth at 20-10°C RTG was reached by copying root diameter composition of plants in 0-5 cm depth grown at 20°C RT and 15°C RT, respectively. Root temperature influenced root diameter composition of the root system and its distribution with depth by affecting root branching and possibly elongation of individual roots as seen in the rhizotrone experiment: Decreased root elongation rate at 10°C RT and favored lateral root growth by temporal temperature change from 20°C to 15°C RT. This stimulation might also be important for root production at the vertical root temperature gradient, i.e. at spatial temperature change.

3.3 Carbon and nitrogen in plants compared at the same age

The observed changes in root morphology of barley plants caused by root temperature have been analyzed for related changes in carbon and nitrogen allocation as these are two of the most important elements in plant metabolism.

3.3.1 Carbon content and partitioning

Total carbon content increased with biomass irrespective of the temperature treatment (Tab. 4, Fig. 15). Highest C content (~ 0.8 g) was reached in plants grown at the vertical root temperature gradient which also reached the highest total biomass. But regarding carbon contents of shoots and roots separately, values indicated an additional process beside dry weight controlling carbon content in roots. A strong linear relation consisted between C content and dry weight in shoots at all temperature treatments (R^2 = 0.94-1.00; Fig. 27a), while less relation occurred in roots (e.g. R^2 = 0.62 at 20-10°C RTG; Fig. 27b).

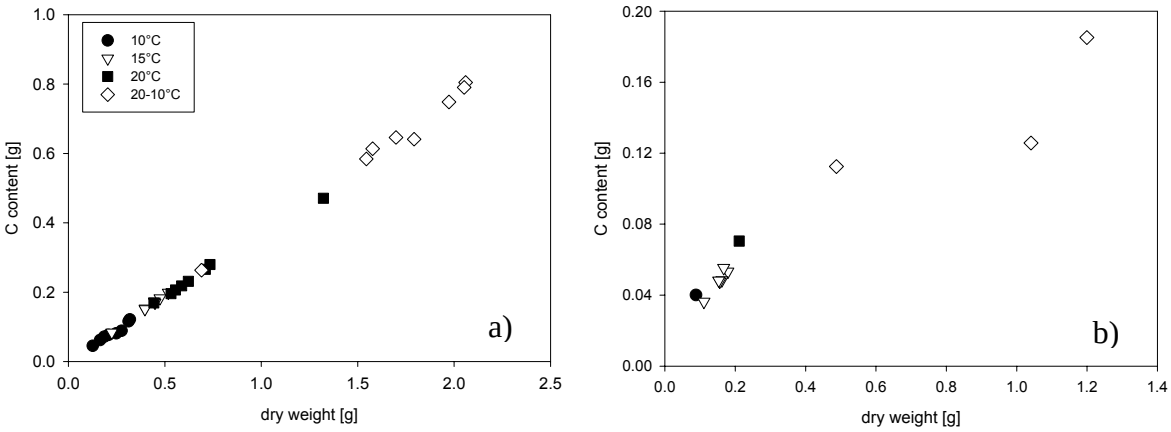

Fig. 27: a) Relation between C content and dry weight in shoots and b) roots. Single plant data are shown. Exceptions are values of 10°C and 20°C RT in b). Here means are shown, because of operational reasons.

C partitioning between roots and shoots indicated that with increasing biomass aboveground the carbon allocation to the root declined (Fig. 28a). The data of C_{root}/C_{shoot} ratio also supported the hypothesis of C allocation controlled by an additional process beside biomass, since these data widely disagreed with those of root/shoot biomass ratio. Only plants grown at 10°C RT showed nearly identical values for both ratios (Fig. 28a, Fig. 16). The C_{root}/C_{shoot} ratios in plants grown at the other temperature treatments were lower than root/shoot biomass ratios, especially in plants grown at 20-10°C RTG. Increasing C allocation to shoots at the

cost of root C content was also described when regarding C concentration within the different plant parts (Fig. 28b). The values obtained for shoot C concentration were nearly the same at all temperature treatments (~ 0.38 g g^{-1} dry weight). In contrast, a lower C concentration in roots was observed in plants grown at the vertical root temperature gradient compared to plants grown at uniform root temperatures (< 0.2 vs. ~ 0.3 g g^{-1} dry weight; Fig. 28b).

	Root temperature [°C]			
Plant part	**10**	**15**	**20**	**20-10**
Total plant	0.123[a]	0.229[b]	0.325[c]	0.799[d]
Shoot	0.083[a]	0.179[b]	0.255[c]	0.637[d]
Root				
total	0.040[a]	0.050[a]	0.070[b]	0.137[c]
0-5 cm	0.021[a]	0.032[b]	0.042[c]	0.093[d]
5-10 cm	0.017[a]	0.012[b]	0.013[ab]	0.042[c]
10-15 cm	0.003[a]	0.002[a]	0.008[b]	0.009[b]
15-20 cm	n.d.	n.a.	0.007	n.d.

Tab. 4: C content [g] in plants grown at different root temperature treatments. Mean is reported. Statistical analysis was performed by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences between the temperature treatments compared in rows; n.d. = not determined; n.a. = not available.

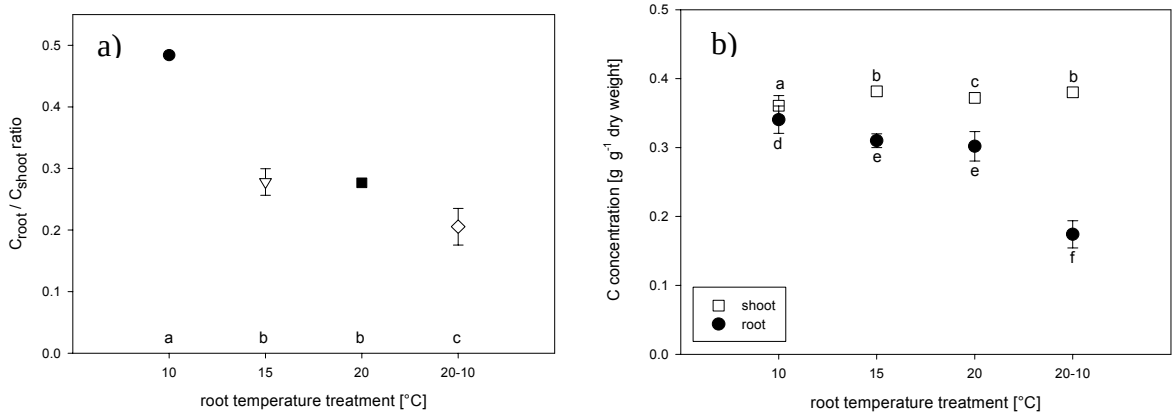

Fig. 28: a) Carbon allocation to root and shoot presented as C_{root}/C_{shoot} ratio and b) C concentration in plants grown at different root temperature treatments. Mean and standard deviation are shown. Statistical analysis was performed by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences.

A closer look on roots in different depths illustrated similar C concentrations in roots: Irrespective of root depth and temperature treatment values ranged between 0.25 g g^{-1} dry weight and 0.35 g g^{-1} dry weight except within 0-5 cm depth (Fig. 29, App. 8). In this depth, plants grown at the vertical temperature gradient reached extremely low concentration (~ 0.17 g g^{-1} dry weight), although C content within 0-5 cm depth was highest of all (Tab. 4). In conclusion, independent of root temperature treatment C allocation within the plant was mainly determined by shoot mass, but results of C content in roots revealed that it has to be determined by an additional process beside biomass. This was especially significant for plants grown at 20-10°C RTG.

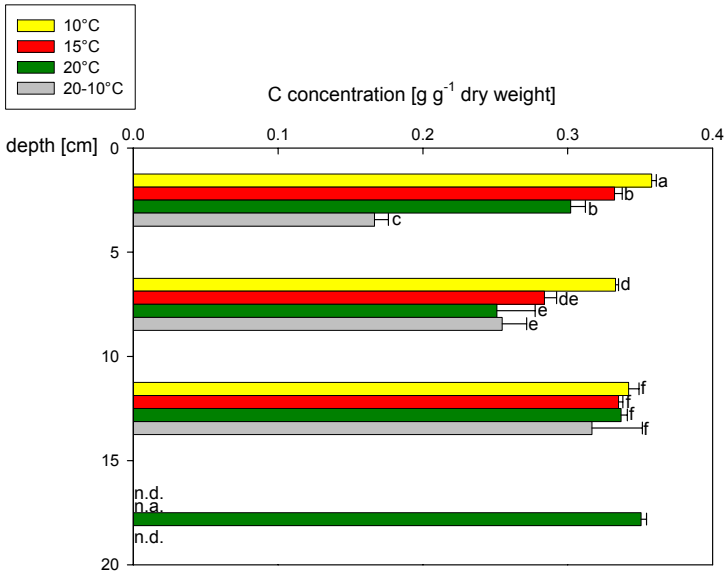

Fig. 29: Carbon concentration in roots at different depths. Mean and standard error of the mean are shown. Statistical analysis was performed by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences. n.d. = not determined, n.a. = not available.

3.3.2 Nitrogen content and partitioning

As seen for total C content, total N content in plants of different temperature treatments seemed to be related to biomass (Tab. 5). But as already observed for the C content, this was attributed to N content in shoots (R^2 = 0.95-1.00 at all treatments; Fig. 30a). The root nitrogen content remained rather constant (e.g. R^2 = 0.62 at 20-10°C RTG, Fig. 30b), although root biomass significantly differed between the temperature treatments (Tab. 5; Fig. 15). Therefore, nitrogen partitioning within the plants did not follow the pattern of root/shoot biomass partitioning. As already seen for C partitioning, only plants grown at 10°C RT reached the same values for N_{root}/N_{shoot} ratio and root/shoot biomass ratio (Fig. 31a, Fig. 16). Plants at the other treatments showed lower values, especially plants grown at vertical root temperature gradient (Fig. 31a).

	Root temperature [°C]			
Plant part	**10**	**15**	**20**	**20-10**
Total plant	0.018[a]	0.035[b]	0.046[c]	0.112[d]
Shoot	0.013[a]	0.029[b]	0.037[c]	0.100[d]
Root				
total	0.006[a]	0.006[a]	0.009[b]	0.009[b]
0-5 cm	0.002[a]	0.004[b]	0.005[b]	0.006[c]
5-10 cm	0.003[a]	0.002[b]	0.002[bc]	0.003[ac]
10-15 cm	0.00046[a]	0.00036[a]	0.001[b]	0.00073[c]
15-20 cm	n.d.	n.a.	0.001	n.d.

Tab. 5: N content [g] in plants grown at different root temperature treatments. Mean is reported. Statistical analysis was performed by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences between the temperature treatments compared in rows; n.d. = not determined; n.a. = not available.

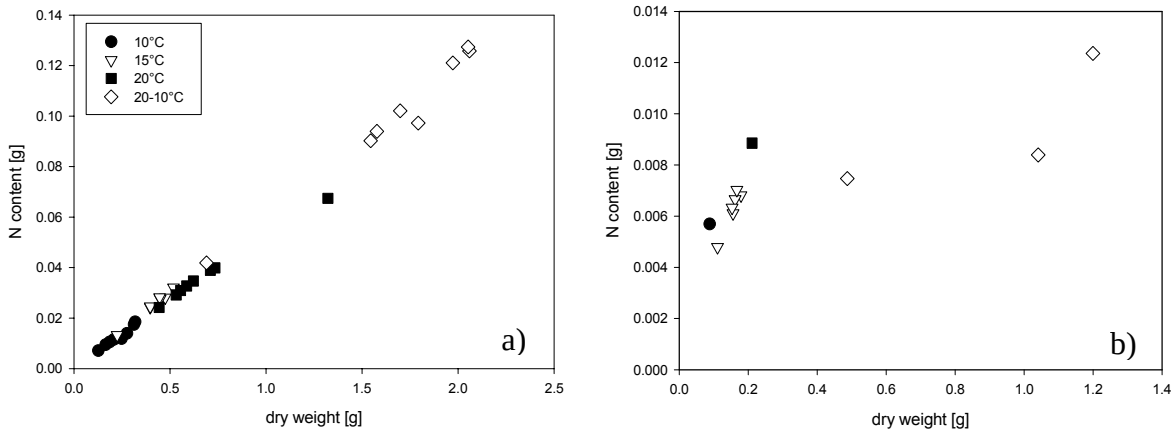

Fig. 30: a) Relation between N content and dry weight in shoots. b) Relation between N content and dry weight in roots. Single plant data are shown. Exceptions are values of 10°C and 20°C RT in b). Here means are shown, because of operational reasons.

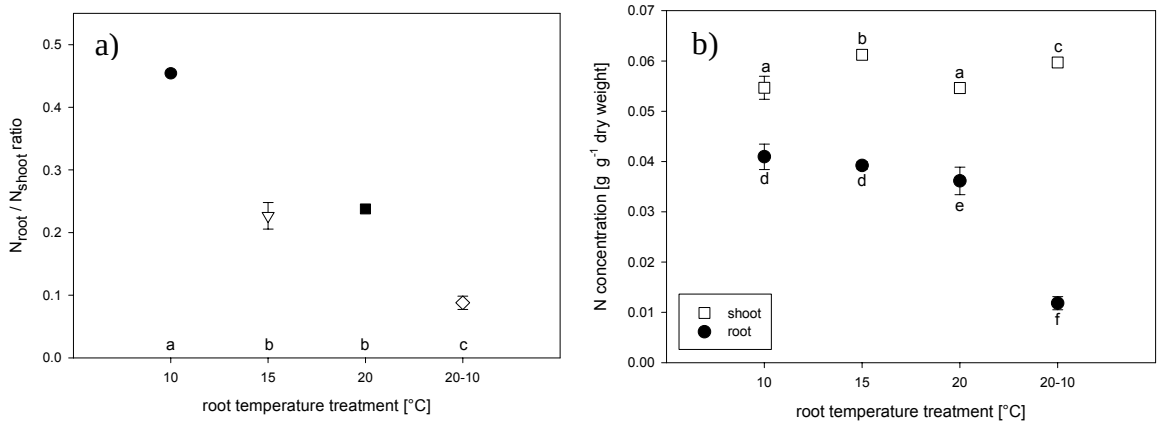

Fig. 31: a) Nitrogen allocation to root and shoot presented as N_{root}/N_{shoot} ratio and b) N concentration in plants grown at different root temperature treatments. Mean and standard deviation are shown. Statistical analysis was performed by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences; $n = 8$.

Consequently, N concentration in roots of plants grown at the vertical root temperature gradient was less than in roots of plants grown at uniform root temperatures (~ 0.01 g g^{-1} vs. ~ 0.04 g g^{-1} dry weight). In contrast, shoot N concentration was at the same level at all temperature treatments (~0.55 - 0.06 g g^{-1} dry weight; Fig. 31b).

Unlikely to distribution pattern of C concentration, significant differences could be noticed between all temperature treatments and depths regarding the distribution pattern of N concentration (Fig. 32). In general, a slight increase in N concentration with increasing depth could be observed at all temperatures (Fig. 32). This effect was however strengthened by decreasing root mass with depth which exceeded the simultaneously decreasing N content (Tab. 5). However, plants reached similar concentration values either in 0-5 cm and 5-10 cm (15°C and 20°C RT) or in 5-10 cm and 10-15 cm depth (10°C RT and 20-10°C RTG; App. 9). Remarkably, the N concentrations within each depth were much lower in plants grown at 20-10°C RTG than in plants grown at uniform root temperatures. This was in contrast to C concentration. There, a difference only occurred in 0-5 cm depth (Fig. 29).

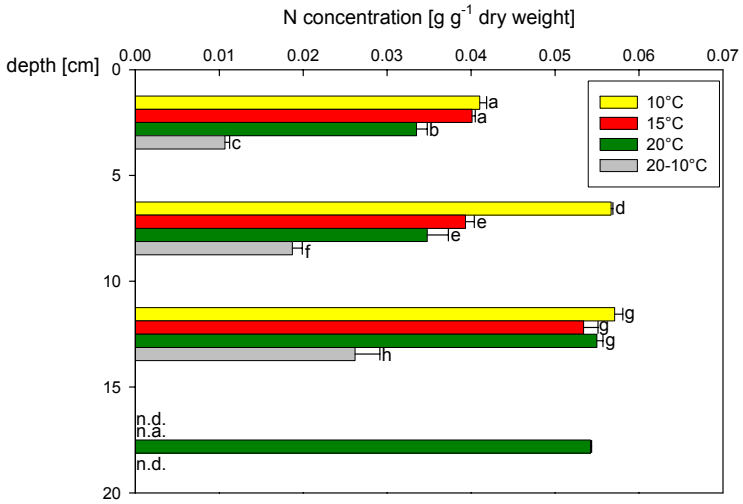

Fig. 32: N concentration in roots at different depths. Mean and standard error of the mean are shown. Statistical analysis was performed by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences; n.d. = not determined, n.a. = not available.

The following C/N ratios resulted out of single element data shown before: C/N ratios in roots were higher than in shoots (Fig. 33). Especially the C/N ratio in roots of plants grown at the 20-10°C RTG treatment was very high (~ 15 vs. ~ 8) due to the extremely low N values. Uniform C/N ratio of plant shoots grown at all temperature treatments and of roots of plants grown at uniform root temperature also corresponded with element data shown before.

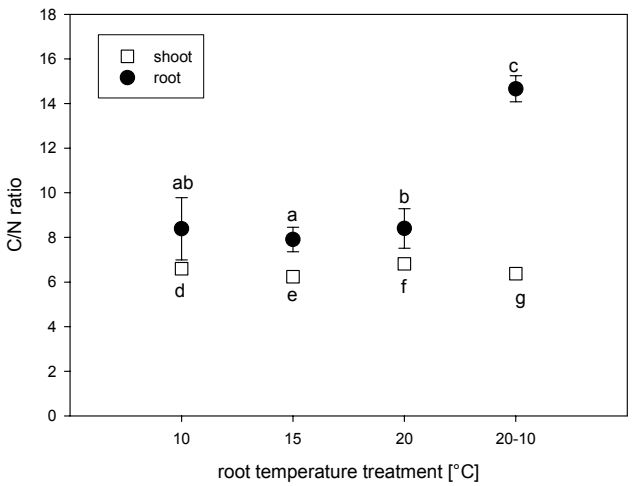

Fig. 33: C/N ratio in shoots and roots. Mean and standard deviation are shown. Statistical analysis was performed by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences.

3.3.3 Nitrogen metabolism

In the previous chapter, the impact of root temperatures on N partitioning within plants was described. Examination of nitrogen fractionations within the plant can give greater insight in the underlying mechanisms. For that purpose, nitrate, free amino acid and protein concentrations were analyzed in plants grown at 20-10°C RTG and 15°C RT. This particular uniform root temperature was chosen, because it represents the average temperature of the

gradient treatment. Therefore it can be expected, that the metabolic compounds behave in the same way at both treatments, if a gradient in root temperature does not affect them.

3.3.3.1 Nitrate in plants

In general, the fraction of NO_3-N on total N was higher in roots than in shoots (here: second developed leaf), irrespective of root temperature (Fig. 34, App. 11). Fractions in roots reached ~40-60 %, while fractions of NO_3-N in shoots ranged between ~10 % and 20 %. In roots of plants grown at 20-10°C RTG fraction of NO_3-N on total N seemed to be reduced, but due to small sample size statistics were not performed.

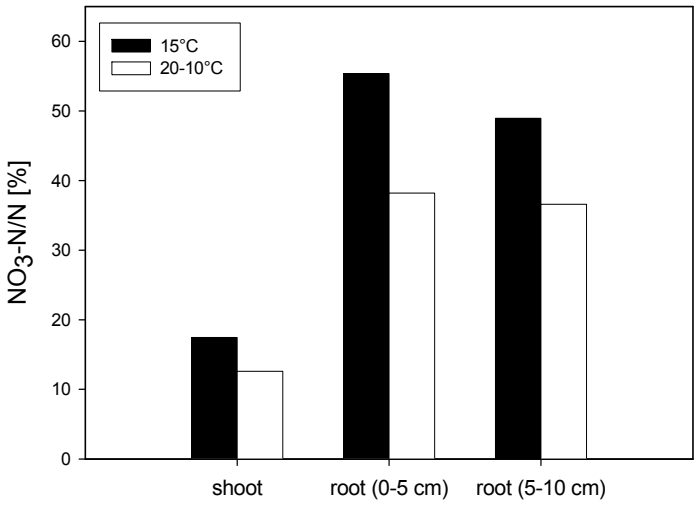

Fig. 34: Fraction of NO_3-N on total N [%] in plants grown at different root temperature treatments. Mean is shown; n = 2.

3.3.3.2 Free amino acids in plants

Independent of root temperature treatment, total free amino acid concentration in shoots (here: second developed leaf) of barley was higher than in roots (Fig. 35). The concentration of total free amino acids in the shoot was doubled in plants grown at 20-10°C RTG compared to plants grown at 15°C RT (Fig. 35). Therefore, most of individual free amino acids attained higher concentrations in shoots of plants grown at 20-10°C RTG. Exceptions were aspartate (ASP) and glutamate (GLU), both showing no concentration differences between the two temperature treatments (Fig. 36, App. 12, 13).

No differences of total free amino acid concentration occurred between roots of different depths and temperatures (Fig. 35). This was also valid for the individual free amino acids (data not shown).

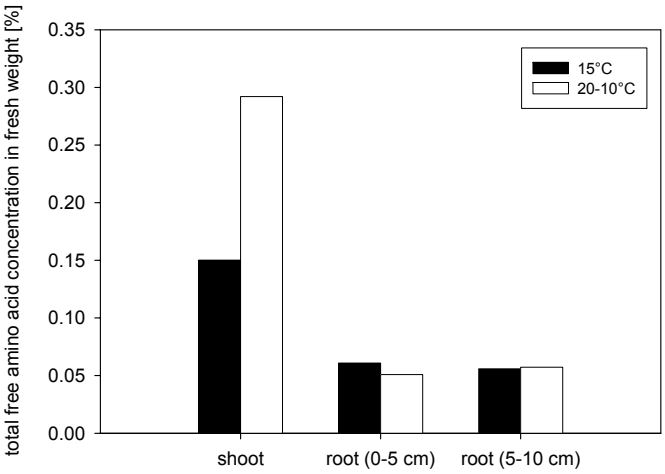

Fig. 35: Concentration of total free amino acids in fresh weight [%] of plants grown at different root temperature treatments. Mean is shown; n = 2.

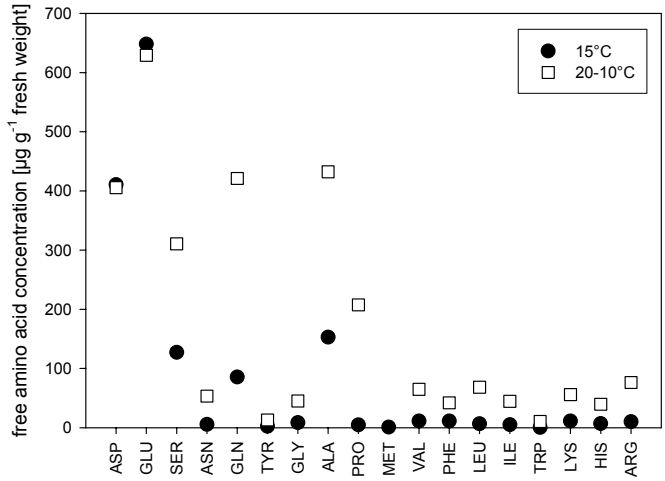

Fig. 36: Concentration of the individual free amino acid [µg g^{-1} fresh weight] in the shoot of plants grown at 15°C RT and 20-10°C RTG. Mean is shown; n = 2.

The $^{15}N/^{14}N$ ratios of the individual free amino acids revealed lower ratios for glutamate (GLU), serine (SER), alanine (ALA) and valine (VAL) in shoots of plants grown at 20-10°C RTG compared to 15°C RT (Fig. 37). That means, the fraction of ^{15}N tracer (cf. Chap. 2.6) was lower in these amino acids at 20-10°C RTG compared to 15°C RT after 9 hours of labeling. This difference and the concentration differences of the individual free amino acids in shoots reported before supported the hypothesis that a vertical gradient in root temperature affects N metabolism.

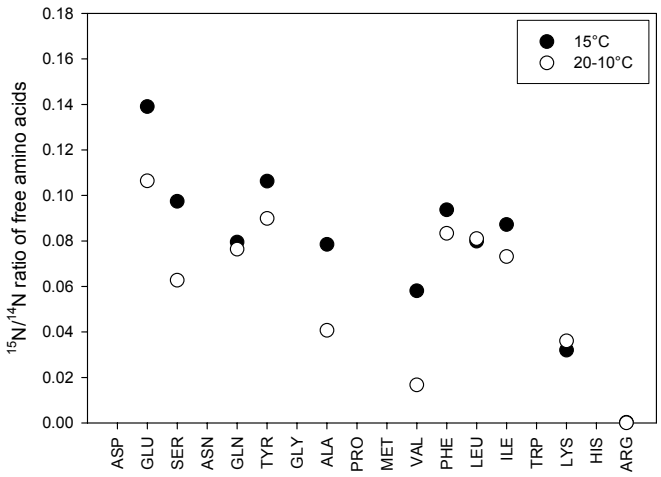

Fig. 37: $^{15}N/^{14}N$ ratio of free amino acids in shoots grown at different root temperature treatments. Mean is shown; n = 2.

3.3.3.3 Proteins in plants

Protein concentration showed some remarkable differences between plants grown at 15°C RT and at vertical root temperature gradient (Fig. 38) supporting the hypothesis of an impact of vertical root temperature gradient on N metabolism. Concentration of proteins did not differ between plant parts of the 15°C RT treatment, while plants grown at 20-10°C RTG reached very low values in roots at 0-5 cm depth. Data of both temperature treatments showed same values in shoots (here: second developed leaf), but a lower concentration in roots at 0-5 cm depth and a higher concentration in 5-10 cm depth, when plants were grown at 20-10°C RTG.

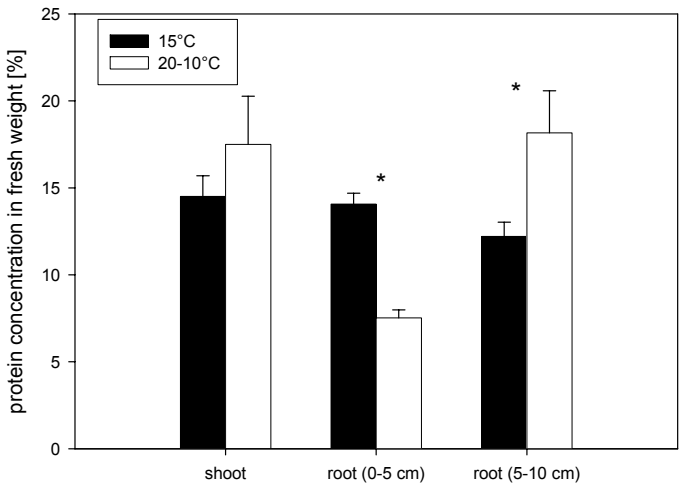

Fig. 38: Protein concentration in fresh weight [%] of plants grown at different root temperature treatments. Mean and standard error of the mean are shown. Statistical analysis was performed by t-test; $p < 0.05$; n = 4; * significantly different.

3.3.4 Summary

Total C and N contents in plants correlated with total biomass, whereas C and N partitioning between roots and shoots was affected by an additional process, promoting element concentrations in shoots at the cost of concentrations in roots. While the distribution pattern of C concentration in roots was widely independent of root temperature, N concentration in roots was more temperature dependent. This was illustrated by reduced N concentrations in each depth at 20-10°C RTG compared to all uniform root temperature treatments. Furthermore, N fractionations in roots of plants grown at 20-10°C RTG differed from those in plants grown at uniform root temperature. This supported the hypothesis that N metabolism is influenced by a vertical gradient in root temperature. Higher total free amino acid concentration was observed in the shoot at 20-10°C RTG when compared with 15°C RT resulting from higher concentrations of nearly all individual free amino acids. The different $^{15}N/^{14}N$ ratios obtained when comparing individual free amino acids in shoots at 15°C RT and 20-10°C RTG indicated variation in the dynamic of N metabolism, as they resulted from differences in transport or transformation of ^{15}N. In roots mainly differences in protein concentration occurred. Protein concentration was extremely low in roots of 0-5 cm depth when grown at 20-10°C RTG.

3.4 Root temperature effects and the variation of plant development stage or age

All previously presented results were obtained for plants of the same age but of different development stages. In this chapter it should be analyzed, whether plant responses to root temperature vary with plant age and development stage. This knowledge also allows deciding, whether effects reported until now resulted from the influence of root temperature or from comparing plants of different development stages. To gain this information, uniform root temperatures of 10°C and 20°C were chosen as experimental temperatures. Based on the results obtained for plants compared at the same age (cf. Chap. 3.1-3.3), it was more likely that comparing plants at these opposite temperatures would result in measurable and significant differences than comparing plants at 10°C and 15°C, for instance. A time series was conducted allowing the comparison of plants of same age but also of same development stage. Additionally, plants were grown at 20°C until they reached the same development stage as plants at 10°C, when harvested after 30 days. These plants were used for more detailed studies about changing effects occurring in root morphology.

3.4.1 Shoot and root mass

Measurements of a time series illustrated, that the previously mentioned root temperature effects on biomass (Chap. 3.1.4) mainly resulted from comparing plants at different development stages:

Plants grown at 10°C and 20°C RT showed a very dynamical pattern in shoot and root biomass production. They always differed in biomass when compared at the same age. In this case, biomass of plants grown at 20°C RT exceeded that of plants grown at 10°C at all times (Fig. 39). In contrast, changing effects were observed within the time series when comparing plants at same development stages. Plants of the same development stage (Fig. 8) differed in shoot mass until reaching day 22 after germination (Fig. 39). Exceeding this age, biomass of plants grown at 10°C RT and 20°C RT became more and more similar, when they were compared at the same development stage. For example, shoot dry weight of plants grown at 20°C and 10°C RT was not significantly different (~ 0.25 g) when both reached development stage 21 (first tiller developed; at day 24 and 30, respectively; n = 8). The same effect of similarities in dry weight was noticed with roots of older plants compared at the same development stage (Fig. 39). This analogy in biomass of plants older than 22 days and compared at the same development stage might be explained by an extremely enhanced RGR between day 22 and 26 (Tab. 6). Plants grown at 20°C RT were harvested before day 26 due to the required development stage and therefore did not experience this increase in RGR. In contrast, biomass production of plants grown at 10°C RT was stimulated by this strong increase in RGR and thus enabled analogies in biomass between both temperature treatments.

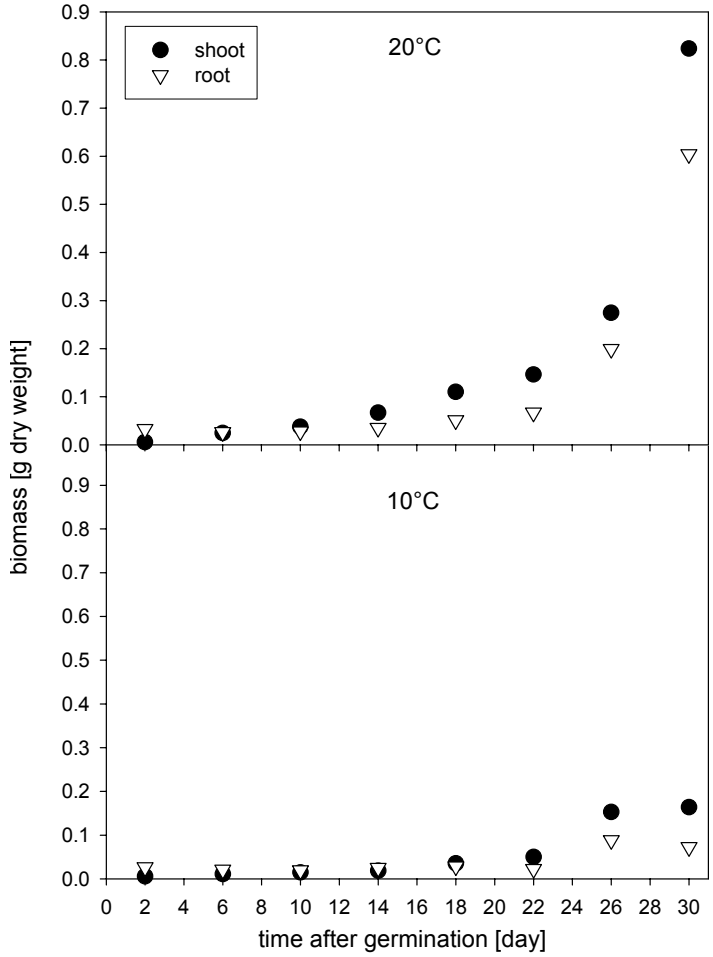

Fig. 39: Time series of shoot and root mass gain of plants grown at 20°C and 10°C uniform root temperature. Mean is shown. Shoot mass = closed circles; root mass = open triangle; n = 2.

Time after germination [d]	10°C		20°C	
	shoot	root	shoot	root
6	15.27	-6.12	37.88	-5.55
10	7.81	-1.53	10.68	1.49
14	5.87	6.14	14.43	5.88
18	15.97	3.24	12.43	9.29
22	**8.69**	**-5.87**	**7.12**	**6.80**
26	**27.96**	**34.30**	**15.74**	**27.30**
30	1.73	-4.93	27.47	27.73

Tab. 6: Relative growth rate of shoot and root [%] at 10°C and 20°C uniform root temperature. Mean is shown; negative data might occur due to small sample number and invasive sampling method: RGR was calculated by data obtained from different plants; n = 2.

As RGR of roots and shoots differed with time and root temperature treatment, biomass allocation to root and shoot also differed. At 20°C RT root/shoot ratio fell below 1 when reaching day 10; and until harvest never exceeded it again. That means, at day 10 more shoot than root mass was present for the first time. At 10°C RT this shift occurred when plants aged 18 days (Fig. 40).

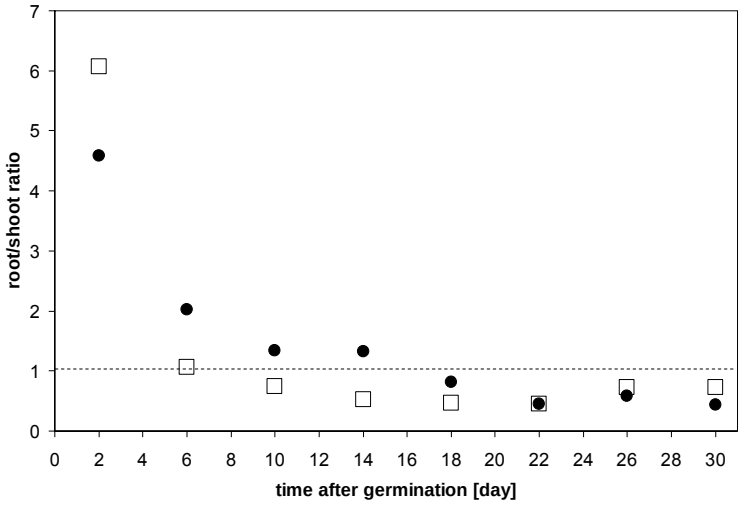

Fig. 40: Time series of root/shoot biomass ratio at 10°C (closed circle) and 20°C (open square) uniform root temperature; n = 2. Dashed line marks change between root or shoot dominance.

In contrast to plant biomass production, root/shoot ratio seemed to be independent of root temperature, plant age and development stage, when plants aged between 22 and 30 days. Neither plants of the same development stage nor of the same age showed strong differences in root/shoot ratio within this timeframe (Fig. 16, Fig. 40), although they were grown at different root temperatures.

3.4.2 Root morphology of plants compared at the same development stage

As already mentioned in Chap. 3.4.1, biomass did not differ between plants grown at 10°C and 20°C RT when older than 22 days and compared at the same development stage. This uniformity also applied to root dry weight distribution with depth, total root length and total root surface area as well as to distribution of root length and root surface area with depth (Tab. 7).

Plant part	10°C			20°C		
	Dry weight [g]	Root length [cm]	Root surface [cm²]	Dry weight [g]	Root length [cm]	Root surface area [cm²]
Total plant	0.32	-	-	0.33	-	-
Shoot	0.23	-	-	0.25	-	-
Root						
total	0.09	436.00	102.99	0.07	449.32	130.72
0-5 cm	0.058	259.23	61.93	0.053	257.38	78.64
5-10 cm	0.025	156.84	35.36	0.016	148.23	41.97
10-15 cm	0.006	22.57	6.48	0.003	43.72	10.10
15-20 cm	0.00012	0.18	0.03	n.a.	n.a.	n.a.

Tab. 7: Biomass and root morphology data of plants grown at 10°C and 20°C uniform root temperature and harvested at same development stage. Mean is reported. No statistically significant differences occurred between the treatments. Statistical analysis was performed by t-test; $p < 0.05$; $n = 8$; n.a. = not available.

Plants older than 22 days and compared at the same development stage showed same pattern of root diameter composition at entire root level as plants compared at the same age (30 days, cf. Chap. 3.2.4): Fraction of thin roots (0.0-1.0 mm diameter) was higher and fraction of thicker roots (1.0-2.0 mm diameter) was lower in plants grown at 20°C RT compared to 10°C RT (Tab. 8, Fig. 23). Additionally, as seen for plants compared at the same age, slightly contrasting distribution patterns of root diameters with depth appeared between the temperature treatments (Fig. 41), although these plants did not differ in one of the other morphological components. Plants grown at 20°C RT showed fewer (5.0 % vs. 8.7 %) thick roots (1.0-2.0 mm diameter) involved in root system formation, but a higher fraction (0.74 % vs. 0.31 %) of roots with 2.0-3.0 mm diameter in 5-10 cm depth compared to plants grown at 10°C RT. Furthermore, the fraction of thin roots (0.0-1.0 mm diameter) was higher in 10-15 cm depth at 20°C RT than at 10°C RT (7.3 % vs. 2.3 %; Fig. 41).

Root diameter [mm]	10°C Fraction on total root length [%]	20°C Fraction on total root length [%]	P
0.0-1.0	73.56	78.06	0.027
1.0-2.0	22.57	16.04	< 0.001
2.0-3.0	2.63	3.00	n.s.

Tab. 8: Root diameter composition at entire root level. Mean is reported. Statistical analysis was performed by t-test; $p < 0.05$; n = 8; n.s. = not significantly different.

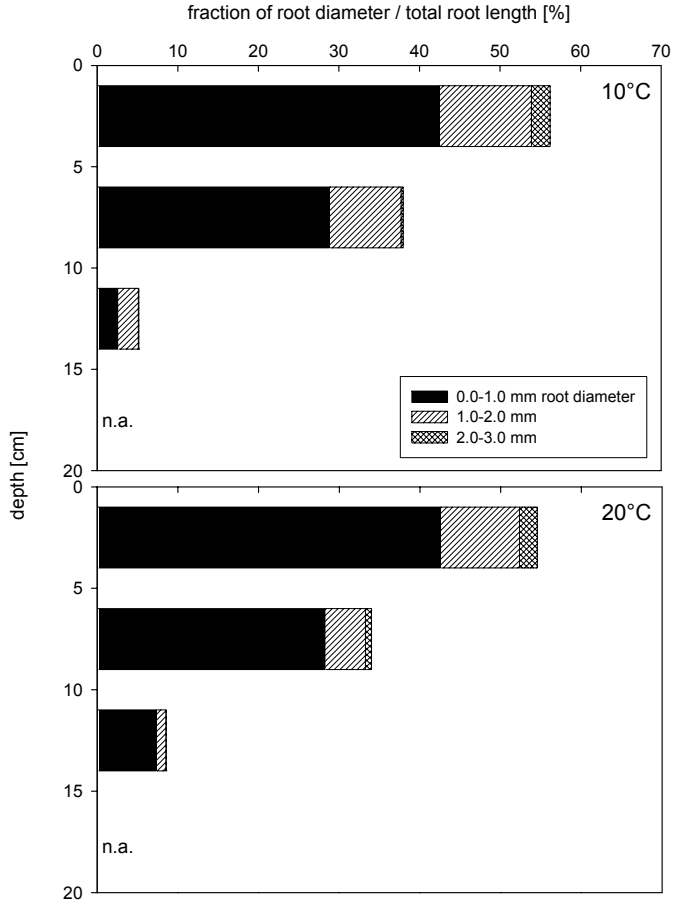

Fig. 41: Fraction of individual root diameters on total root length distributed with depth. Plants were of same development stage and were grown at 10°C and 20°C uniform root temperature; n = 8; n.a. = not available.

3.4.3 C and N in plants compared at the same development stage

The same effects as stated for plants compared at the same age (Chap. 3.3) occurred comparing C and N data of plants of same development stage and older than 22 days (Tab. 9). No significant differences were noticed between shoot C concentrations of plants grown at 10°C and 20°C RT. In roots concentration values ranged between 25 % and 35 % in each depth, as already seen when plants were compared at the same age. Even C partitioning

between root and shoot showed same tendencies (0.48 vs. 0.22 at 10°C and 20°C RT, respectively).

	C concentration [g g^{-1} dry weight]			N concentration [g g^{-1} dry weight]		
Plant part	**10°C**	**20°C**	**P**	**10°C**	**20°C**	**P**
Shoot	0.36	0.37	n.s	0.055	0.057	n.s.
Root						
total	0.34	0.28	< 0.001	0.041	0.018	< 0.001
0-5 cm	0.36	0.27	< 0.001	0.041	0.016	< 0.001
5-10 cm	0.33	0.32	n.s	0.057	0.023	< 0.001
10-15 cm	0.34	n.a.	n.d.	0.057	n.a.	n.d.
15-20 cm	n.d.	n.a.	n.d.	n.d.	n.a.	n.d.

Tab. 9: C and N concentration [g g^{-1} dry weight] in plants of same development stage and grown at 10°C and 20°C uniform root temperature. Mean is reported. Statistical analysis was performed by t-test; n.s. = not significantly different; n.d. = not determined; n.a. = not available.

Differences in root N concentration were identified, while shoot N concentration was the same at both temperature treatments (Tab. 9). N concentration in roots determined with depth perfectly corresponded in pattern with the one seen comparing plants of the same age (Fig. 32), although here biomass did not differ (Tab. 7). N partitioning between root and shoot also reached a higher value at 10°C compared to 20°C (0.45 and 0.09, respectively), as already seen when comparing plants of the same age (Fig. 31a).

3.4.4 Summary

Differences in biomass of younger plants grown at 10°C RT and 20°C RT occurred, no matter whether plants were compared at same development stage or age. They were caused by stimulated plant growth at higher root temperatures. This difference was still evident in older plants compared at the same age, but disappeared comparing plants of the same development stage but different age between day 22 and day 30 after germination. Root/shoot biomass ratio

of older plants (22-30 days) was nearly the same at both temperature treatments. However, this was independent of the comparison characteristic (development stage or age) chosen.

The same amount of root dry weight at the same development stage resulted in similarities of root surface area and root length as well as of their distribution with depth when comparing plants grown at 10°C RT and 20°C RT. Despite of this fact, root diameter composition (fractions of individual root diameters on the analyzed root system) differed. At the entire root level, the same pattern of root diameter composition was observed for the respective root temperature treatment, as already seen for plants compared at the same age (30 days). The diameter distribution with depth also differed between the temperature treatments. Results of C and N analyses were comparable to those of plants compared at the same age, although they did not differ in biomass. These findings supported the hypothesis that an additional process beside biomass has to control C and N allocation in barley plants (cf. Chap. 3.3).

3.4.5 Conclusions for interpretation of results obtained with plants compared at the same age

In any case, differences observed for C and N concentrations and partitioning in the plants as well as for root diameter composition can be considered as root temperature effects when comparing older plants either at the same development stage or age. Significant C and N patterns for each root temperature seemed to be committed independent of plant age and development stage at least when plants were older than 22 days. The same effect was observed for root diameter composition on entire root level. Fractions of individual root diameters at different depths varied with plant age/development stage, however differences between the root temperature treatments occurred in any case. Furthermore, the pattern of root mass distribution with depth seemed to be independent of plant age and development stage, since similarities in root mass fractions at different depth occurred either between plants of the same development stage (10°C RT vs. 20°C RT, Tab. 7) or of the same age (10°C RT vs. 15°C RT vs. 20-10°C RTG, App. 1a). That means, root temperature has to be the most important parameter determining root mass distribution with depth, root diameter composition and C as well as N concentration and partitioning in barley plants analyzed in this study. As root length and surface area are related to root diameter compositions, the observed differences in root morphology of plants compared at the same age (30 days) also resulted from root temperature differences, but in a more indirect way.

Therefore, all effects observed with plants grown at the vertical gradient in root temperature can be considered as effects caused by root temperature. However, one has to be aware that they occur in changing amplitudes during the plant life cycle.

3.5 Nutrient uptake

Root temperature effects on plant development and growth velocity as well as on root morphology (e.g. root length) and plant internal carbon and nitrogen status were presented in the previous chapters. Now, the impact of these structural changes on functional nutrient uptake was analyzed by observing nitrogen and magnesium uptake.

3.5.1 Nitrogen uptake

3.5.1.1 N uptake affected by root temperature

Data of nitrogen uptake kinetics obtained for barley plants in hydroponics (*nutrient uptake kinetics experiment*, Chap. 2.5) showed that ^{15}N label accumulated in shoots of all temperature treatments (excluding 20-10°C RTG as it was not possible to generate the respective gradient due to technical reasons) already ¼ h after labeling (Fig. 42) and increased with time. However, increase in fraction of ^{15}N on total N in shoots of plants grown at 10°C RT was lower than in plants grown at 15°C and 20°C RT. That means, either total ^{15}N uptake by roots was reduced and/or ^{15}N allocation to the shoot was reduced at 10°C RT. Results of ^{15}N partitioning between root and shoot indicated the same partitioning at all temperatures and the same changes in partitioning pattern with time (Tab. 10). This clearly indicated that allocation between root and shoot was not changed by low root temperature, rather nutrient uptake was affected. This was confirmed by data of total ^{15}N content within the plants (Tab. 11). Higher ^{15}N content in plants meant higher total uptake of ^{15}N resulting in higher ^{15}N fractions on total N, since biomass as well as total N concentration [% dry weight] did not significantly differ between the treatments (App. 14). Furthermore, root structure was similar at all treatments due to the same pretreatment of all plants within this experiment,

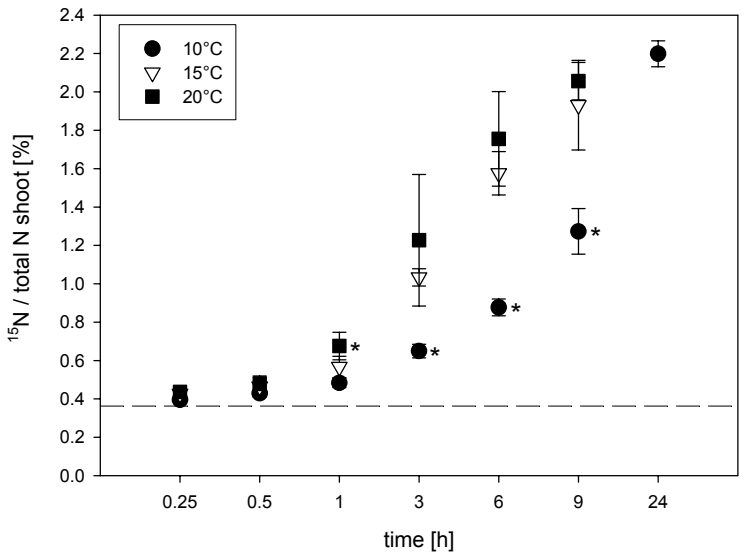

Fig. 42: Kinetic of ^{15}N fraction on total N [%] in shoots of plants labeled at different root temperatures. Mean and standard deviation are shown. Missing data of 24h labeling at 15°C and 20°C are due to analytical difficulties. Dashed line marks natural ^{15}N/N ratio in plants. Statistical analysis was performed by one way ANOVA; $p < 0.05$; n = 3; * significantly different.

	$^{15}N_{root}$ / $^{15}N_{shoot}$ ratio at time [h]							
Root temperature [°C]	**0.25**	**0.5**	**1**	**3**	**6**	**9**	**24**	**48**
10	0.87	0.90	0.85	1.04	1.21	1.34	n.d.	n.d
15	0.87	0.93	0.97	1.11	1.13	n.d	n.d	n.d
20	n.d	0.90	0.91	1.05	n.d	n.d	n.d	n.d

Tab. 10: ^{15}N partitioning in plants at different times and root temperature treatments; mean is shown; missing data are due to analytical difficulties; n = 3; n.d. = not determined.

	^{15}N content [mg] of total plant at time[h]							
Root temperature [°C]	**0.25**	**0.5**	**1**	**3**	**6**	**9**	**24**	**48**
10	0.03	0.03	0.02	0.03	0.05	0.09	n.d.	n.d
15	0.03	0.03	0.03	0.07	0.10	n.d	n.d	n.d
20	n.d	0.04	0.04	0.07	n.d	n.d	n.d	n.d

Tab. 11: ^{15}N content [mg] of the total plant at different times and root temperature treatments; mean is shown; missing data are due to analytical difficulties; n = 3; n.d. = not determined.

The same effect was expected to be seen in sand grown plants of same biomass (App. 15) and root structure (SD of root length = ± 20 %) when they were exposed to different root temperature treatments during labeling. Indeed, plants exposed to 20°C RT for labeling and harvested after 9 h of labeling also showed higher ^{15}N/total N fraction than plants labeled at 10°C RT (2.06 % ± 0.1 vs. 0.99 % ± 0.13; Fig. 43a, open symbols). In contrast, plants exposed to 15°C RT showed lower values than in the *nutrient uptake kinetics experiment* (1.23 % ± 0.13 vs. 1.93 % ± 0.23). This was probably due to the abrupt temperature change of the root system from room temperature (22°C ± 1) to experimental temperature. However, plants exposed to 20-10°C RTG showed similar values as plants at 15°C RT (1.38 % ± 0.17). In plants labeled at 10°C, 15°C RT and 20-10°C RTG minor differences in ^{15}N fraction on total N in the shoot occurred (Fig. 43a, open symbols) despite same ^{15}N content (Fig. 43b, open symbols) due to a slight variation in N concentration (App. 16)

3.5.1.2 N uptake affected by root temperature and structure

No significant difference was seen between all uniform root temperature treatments observing the effect of root structure combined with root temperature on nitrogen uptake (Fig. 43a, closed symbols). No significant difference between 10°C RT and the other uniform root temperatures was detected anymore, when plants were morphologically adapted to the respective root temperature (Fig. 42). A slightly decreased value in plants grown at vertical root temperature gradient was observed (Fig. 43a, closed symbols), although ^{15}N content in shoots was highest in these plants (Fig. 43b, closed symbols).

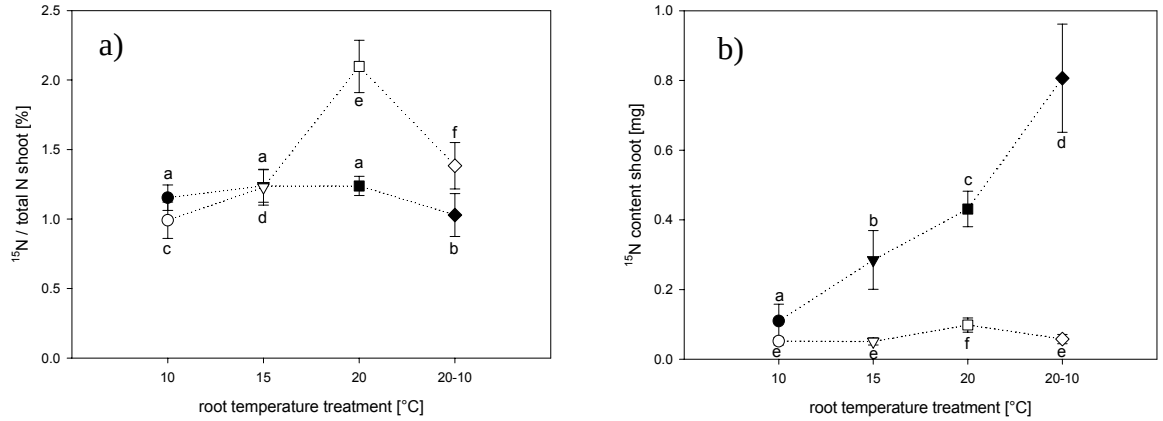

Fig. 43: a) Fraction of ^{15}N on total N [%] and b) ^{15}N content [mg] in shoots of 30 days old plants after 9 h of labeling when labeled in 1 cm depth. Plants germinated and grew at different root temperatures (closed symbols) or were exposed to different root temperatures at day 30 (open symbols). Mean and standard deviation are shown. Statistical analysis was performed by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences.

The same principle of results was gained after adding label in 6 cm depth (Fig. 44a+b). Nevertheless, slight differences were detectable: (1) Plants of same biomass and root structure showed comparable results at 10°C RT and 20-10°C RTG (Fig. 44a; open symbols). (2) Plants morphologically adapted to different temperatures reached a lower value at 10°C RT than at the other uniform root temperature treatments (Fig. 44a; closed symbols). (3) ^{15}N content in shoots at 15°C and 20°C RT was comparable in plants adapted to root temperatures (Fig. 44b) due to equal shoot mass.

In conclusion, N uptake was lower at 10°C RT compared to the other root temperature treatments, but structural differences in plants (e.g. biomass and root morphology) adapted to the respective root temperature overrode this direct temperature effect. Therefore, no differences in enriched nitrogen concentration were observed between the temperature treatments, when plants were germinated and grown at these temperatures. This effect was independent of root compartment involved in uptake.

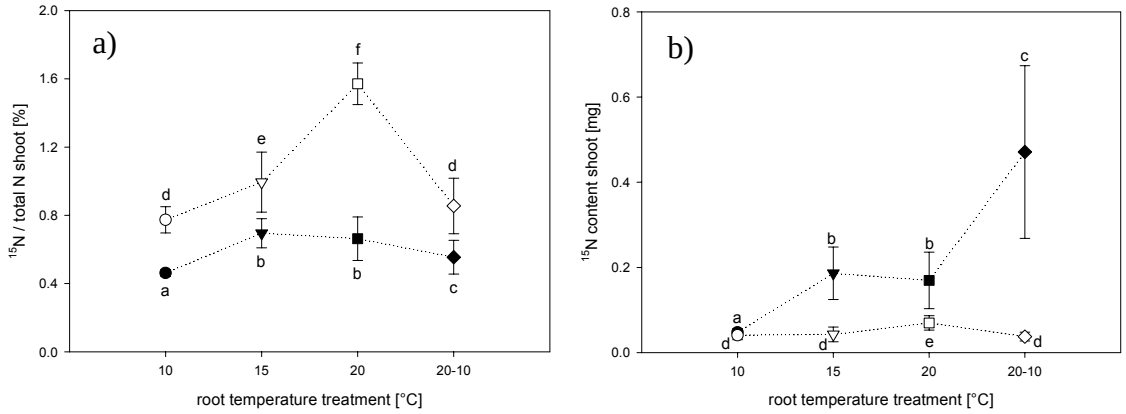

Fig. 44: a) Fraction of ^{15}N on total N [%] and b) ^{15}N content [mg] in shoots of 30 days old plants after 9 h of labeling when labeled in 6 cm depth. Plants germinated and grew at different root temperatures (closed symbols) or were exposed to different root temperatures at day 30 (open symbols). Mean and standard deviation are shown. Statistical analysis was performed by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences.

3.5.2 Magnesium uptake

3.5.2.1 Mg uptake affected by root temperature

Results of the magnesium *uptake kinetics experiment* showed the first tracer accumulation in shoots after 9 h of labeling. The positive correlation of ^{25}Mg/total Mg and increasing uniform root temperature became more distinct with time (Fig. 45). Data of ^{25}Mg content in the entire plant showed the highest total uptake for plants labeled at 20°C RT after 24 h and 48 h (Tab. 12). At this time no significant difference was observed between the content in plants examined at 20°C and 15°C RT (0.081 mg and 0.077 mg, respectively). In contrast, differences between plants labeled at 10°C and 15°C RT at 9 h and 24 h (0.045 mg vs. 0.077 mg and 0.076 mg vs. 0.112 mg, respectively) became negligible after 48 h of labeling (Tab. 12). Apart from variation in total ^{25}Mg content, the partitioning between root and shoot was affected by root temperatures. While this ratio remained ≥ 1 at 10°C and 15°C RT as soon as label appeared in shoots, it decreased with time at 20°C RT (Tab. 13). These results suggested slight root temperature influence on uptake as well as on transport of ^{25}Mg, because biomass and root structure of plants were the same at all temperature treatments (App. 14).

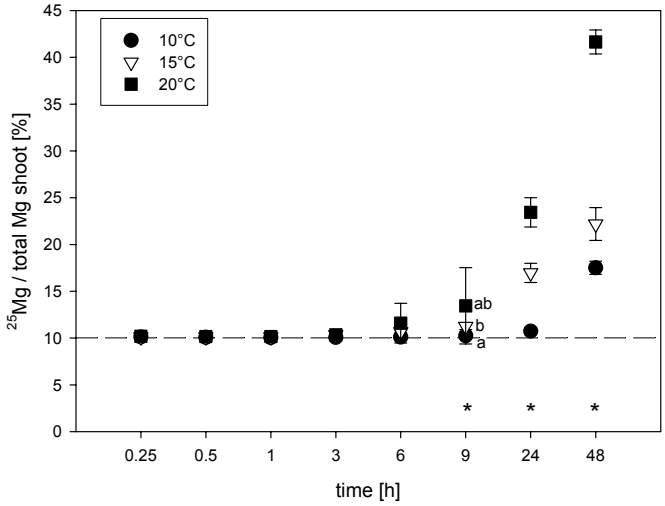

Fig. 45: Kinetic of ^{25}Mg fraction on total Mg [%] in shoots of plants labeled at different root temperatures. Mean and standard deviation are shown. Dashed line marks natural ^{25}Mg/Mg ratio in plants. Statistical analysis was performed by one way ANOVA; $p < 0.05$; n = 3; different letters and * mark statistically significant differences.

Comparable results were obtained analyzing Ca uptake and allocation to root and shoot at 10°C and 20°C RT after 24 h of labeling. The stable isotope ^{44}Ca used as tracer by substituting 50 % of $^{40}Ca(NO_3)_2$ in Hoagland solution did not accumulate in shoots of plants labeled at 10°C but was measured in significant quantities at 20°C RT (3.51 % ± 0.15 ^{44}Ca/total Ca and 8.11 % ± 0.35, respectively). Plants labeled at 20°C RT reached slightly higher ^{44}Ca content at entire plant level than plants labeled at 10°C RT (0.07 mg vs. 0.06 mg; control: ~0.01 mg). These findings indicated root temperature influence on Ca uptake and more distinct on partitioning within the plant.

	^{25}Mg content [mg] total plant at time[h]							
Root temperature [°C]	**0.25**	**0.5**	**1**	**3**	**6**	**9**	**24**	**48**
10	0.06	0.09	0.06	0.04	0.06	0.04	0.08	0.10
15	0.07	0.06	0.06	0.06	0.06	0.07	0.11	0.11
20	0.06	0.08	0.08	0.07	0.08	0.08	0.14	0.22

Tab. 12: ^{25}Mg content [mg] of the entire plant at different times and root temperature treatments; mean is shown; n = 3.

Root temperature [°C]	$^{25}Mg_{root}$ / $^{25}Mg_{shoot}$ ratio at time [h]							
	0.25	**0.5**	**1**	**3**	**6**	**9**	**24**	**48**
10	0.90	1.92	1.56	0.95	2.29	0.79	1.95	1.23
15	1.68	1.65	1.45	1.68	1.64	1.55	1.50	0.99
20	0.91	0.89	1.17	1.06	0.93	0.55	0.35	0.13

Tab. 13: ^{25}Mg partitioning in plants at different times and root temperature treatments; mean is shown; n = 3.

In contrast to results of ^{15}N uptake, no possible reaction due to abrupt temperature change was noticed comparing data of ^{25}Mg/total Mg after 9 h of labeling measured in the *uptake kinetics experiment* and of plants grown in sand and only exposed to different root temperatures for the labeling period (Fig. 45; Fig. 46a, open symbols). In both experiments a slight increase in ^{25}Mg/total Mg was observed at increasing uniform root temperature. Therefore, it was permitted to exclude variation in reaction at vertical root temperature gradient due to abrupt temperature change at first labeling time, although uptake kinetics could not be measured.

3.5.2.2 Mg uptake affected by root temperature and structure

The impact of root structure and root temperature on Mg uptake was examined. No differences were observed between plants only exposed to different root temperatures at labeling (Fig. 46a, open symbols) and plants directly geminated and grown at the specific root temperatures (Fig. 46a, closed symbols). Nevertheless, ^{25}Mg/total Mg in shoots of plants morphologically adapted to 20°C RT was slightly lower compared to all other temperature treatments (Fig. 46a, closed symbols), although ^{25}Mg content in the shoot was higher than in plants grown at the other uniform root temperature treatments (Fig. 46b, closed symbols).

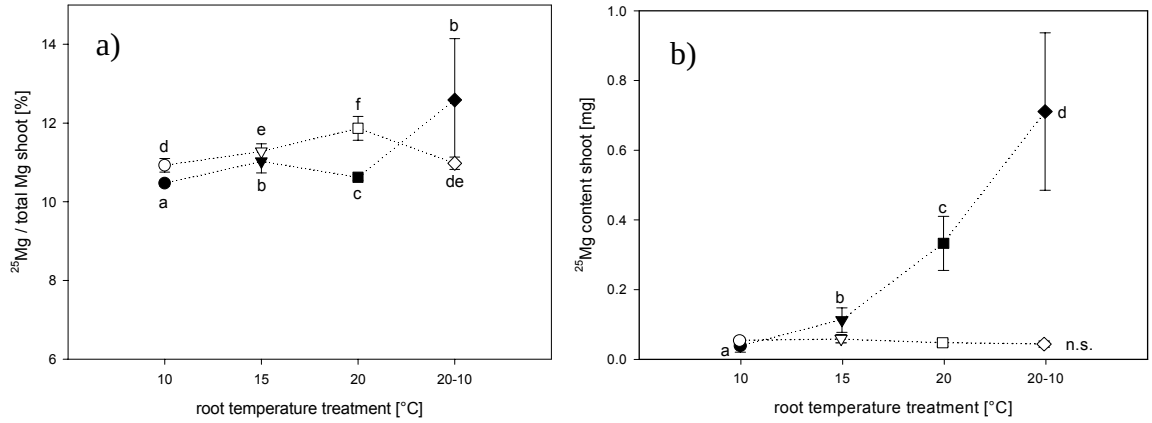

Fig. 46: a) Fraction of ^{25}Mg/total Mg [%] and b) ^{25}Mg content [mg] in shoots of 30 days old plants after 9 h of labeling when labeled in 1 cm depth. Plants germinated and grew at different root temperatures (closed symbols) or were exposed to different root temperatures at day 30 (open symbols). Mean and standard deviation are shown. Statistical analysis was performed by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences; n.s. = not significantly different.

Similar results as described above were obtained, when plants were labeled in 6 cm depth (Fig. 47a), although a slight difference occurred in ^{25}Mg content of plants adapted to 20°C root temperature. They reached similar values as plants adapted to 15°C RT 9 h after labeling (added in 6 cm depth); probably due to their equal shoot mass (Fig. 47; App. 17).

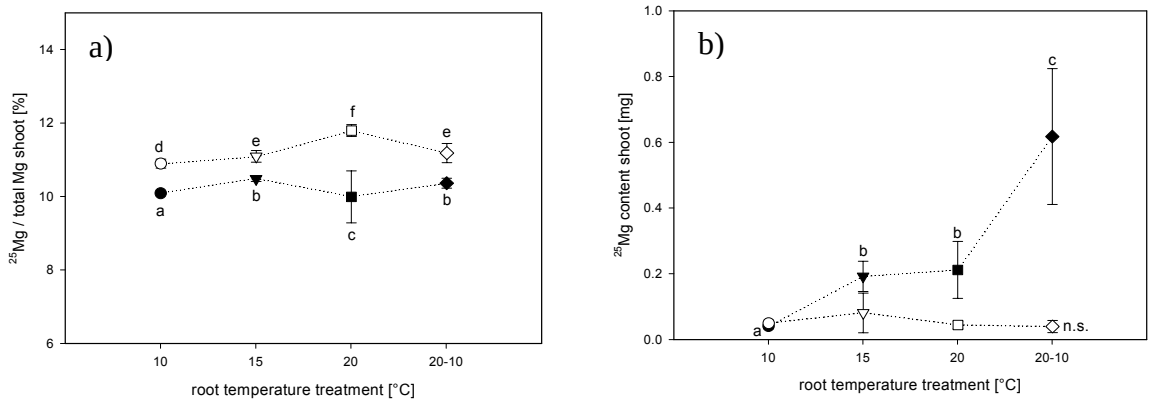

Fig. 47: a) Fraction of ^{25}Mg/total Mg [%] and b) ^{25}Mg content [mg] in shoots of 30 days old plants after 9 h of labeling when labeled in 6 cm depth. Plants germinated and grew at different root temperatures (closed symbols) or were exposed to different root temperatures at day 30 (open symbols). Mean and standard deviation are shown. Statistical analysis was performed by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences, n.s. = not significantly different.

3.5.3 Summary

Experiments conducted to achieve information about the influence of root temperature and root structure on nutrient uptake led to the following results: (1) Low root temperature reduced N as well as Mg uptake. (2) Mg tracer transport from the roots to the shoot was slower than N tracer transport, independent of root temperature. (3) N allocation was not influenced by root temperature, whereas Mg partitioning within the plant differed between the root temperature treatments. Higher root temperatures favored Mg allocation to the shoot. This result obtained for Mg was also valid for Ca. (4) When plants were morphologically adapted to the respective root temperatures, these effects on plant structure overrode direct temperature effects resulting in comparable N concentrations in shoots of all temperature treatments. In contrast, for passive nutrient uptake (Mg) this overriding of effects was not detectable. (5) Nutrient uptake was independent of the root compartment dominating it. No difference in pattern of enriched nutrient fraction in shoots was observed comparing nutrient uptake out of two different depths.

4 Discussion

For the first time, in the present study the influence of spatial soil temperature differences on single plants was examined under controlled conditions as close as possible to nature. The specially developed experimental setup used in this study guaranteed that soil temperature was the only abiotic factor which could cause changes in plant behavior (cf. Chap. 2.2). Results revealed that plants respond in a different way to vertical temperature gradients in soil than to uniform soil temperatures.

It was shown that barley development and growth was accelerated by a vertical soil temperature gradient. At first, this effect will be discussed at entire plant level (Chap. 4.1). Impacts on biomass as well as on carbon and nitrogen allocation to shoot and root will be analyzed in this context, since these variables underlie plant growth and development.

Morphological differences appeared when plants were grown at vertical soil temperature gradients compared to uniform root temperatures. As plants benefit in growth at the vertical soil temperature gradient, especially the detected plasticity in root system was discussed in detail, since the root system is responsible for belowground resource acquisition and also influences translocation to the shoot (Chap. 4.2).

Whether improved resource acquisition may be responsible for enhanced plant growth and whether this may be due to direct root temperature effects on nutrient uptake and translocation or to changed plant morphology will be discussed in Chap. 4.3.

Furthermore, for the first time it was shown that responses of barley plants to soil temperature changed in amplitude with varying plant age and development stage. This will be discussed in Chap. 4.4.

4.1 Vertical temperature gradients in soil cause responses at whole plant level

4.1.1 Variation in time until germination

Several studies have shown previously that time until germination is influenced by soil temperature (McMaster, 2005; Beauchamp & Lathwell, 1967). In general, increasing soil temperature accelerates germination until optimal temperature - depending on plant species - is exceeded (Daws *et al.*, 2002; Wagenvoort & Bierhuizen, 1977). Data obtained in this study

confirmed this result (Fig. 11, p. 31). Within the temperature range considered for vegetative growth of spring barley (Bowen, 1991; Briggs, 1978) time until germination decreased with increasing uniform substrate temperatures (10°C > 15°C > 20°C RT). Time until germination was nearly doubled at 10°C RT compared to 20°C RT (Fig. 11, p. 31). This indicated same relation of processes involved in germination (e.g. cell development and elongation as well as enzymatic reactions during embryonic growth) with substrate temperature as in general common for most biological processes with temperature (Q_{10}~ 2). No difference in time until germination occurred between plants grown at 20°C RT and 20-10°C vertical root temperature gradient due to equal substrate temperatures at sowing depth of 1 cm (20°C at both treatments).

4.1.2 Accelerated plant development

Plant development can be described as the variation in number (not size) of plant organs or as the time needed until a particular phenological stage (e.g. flowering) is reached (Atkinson & Porter, 1996). It is accelerated by increasing uniform root temperature (Sharratt, 1991; Power *et al.*, 1970; Beauchamp & Lathwell, 1967). This was also confirmed for barley development in the present study. A higher number of leaves and tillers were produced with time as well as less time was needed to reach the next development stage at 15°C and 20°C relative to 10°C RT (Fig. 13, p. 33; Fig. 12, p. 32). Similar results were obtained with *Zea mays* by Warrington & Kanemasu (1983) and Macduff et al. (1986) reporting increased number of leaves respectively tillers with increasing root temperature.

The correlation between earlier and/or enhanced leaf emergence and increasing soil temperature is explained by soil temperature directly influencing the meristematic region of the shoot (Engels & Marschner, 1990; Takamura *et al.*, 1961; Beauchamp & Lathwell, 1967). Especially in crop monocots, this region is situated some centimeters below soil surface during the period of leaf initiation and early growth (Shaykewich, 1995; Beauchamp & Lathwell, 1967). Therefore, either formation of leaf primordia initiated early in crop growth or leaf emergence from primordia may be affected by soil temperature (Kirby, 1995; Peacock, 1975). Changing rate of cell division and/or the deployment of cells produced by the meristem can be the underlying mechanisms of variations in leaf emergence (Francis & Barlow, 1988).

However, the results obtained for barley grown at the vertical root temperature gradient were contradictory to this hypothesis. The development of plants grown at the vertical root

temperature gradient was most accelerated with respect to all temperature treatments examined in the current study. The total number of leaves and tillers was highest, although root temperatures influencing the meristematic region of the shoot were the same at 20°C RT and 20-10°C RTG grown plants. Shoot meristems at both root temperature treatments were exposed to 20°C. Therefore, soil temperature has to affect leaf emergence by an additional mechanism besides directly influencing cell division and/or cell deployment in the shoot meristem. Variation in day length (Kirby, 1995) could be excluded as possible factor determining rate of leaf emergence in this study, but plant internal signaling e.g. via phytohormones (Bowen, 1991) influenced by root temperature might be considered as an additional mechanism responsible for variation in leaf emergence. Furthermore, a better nutrient supply of plant shoots might also explain the accelerated development at 20-10°C RTG. A better nutrient supply might be due to variation in plant morphology and/or physiology caused by the vertical root temperature gradient. This hypothesis will be discussed in more detail in the following chapters.

4.1.3 Benefits in plant growth

Plant growth, defined as irreversible increase in dry weight (Atkinson & Porter, 1996) depending on cell division and cell elongation (Lambers *et al.*, 1998), is accelerated by increasing soil temperature. This was observed within temperature optimum ranges (cf. Chap. 1.2) of several plant species (Power *et al.*, 1970; DeLucia *et al.*, 1992; Clarkson *et al.*, 1992; Matthews & Hayes, 1982). Less root growth of barley was detected in the present study at 10°C uniform root temperature compared to 20°C. This was in agreement with results by Briggs (1978), whereas the common assumption of maximum barley root growth at 15°C root temperature (Power *et al.*, 1970) could not be confirmed (Fig. 15, p. 35). This might be explained by differences in the experimental setup. Power et al. (1970) used plants all pre-grown at 15°C before exposing to the respective experimental temperatures. Therefore, plants examined at 15°C root temperature were already adapted to this temperature while plants investigated at 9°C and 22°C root temperature might be limited in growth due to sudden root temperature changes.

Plants grown at 20-10°C vertical root temperature gradient reached maximum dry weight compared to plants grown at uniform root temperatures in the present study. This was not necessarily expected; rather it was more likely that dry weight would be the average of plants grown at 10°C RT and 20°C RT due to the temperature range chosen as gradient. However,

this result was also obtained when another temperature gradient was chosen (15-3.5°C; App. 20). Therefore, it can be stated that plant development as well as dry weight gain is stimulated by vertical root temperature gradients relative to uniform root temperatures, independent of the range of the temperature gradient.

Within the particular temperature optimum range of plant species, plants in general invest less into root biomass production compared to shoot biomass production (Bowen, 1991; Equiza *et al.*, 2001; Davidson, 1969; Engels, 1994; Boucher *et al.*, 2001) and do not differ in root/shoot biomass ratio (Kleier *et al.*, 2001; Davidson, 1969; Pettersson, 1995). The same effects were found in this study and caused root/shoot biomass ratios < 1. No significant differences were obtained when plants were grown at uniform root temperature treatments (Fig. 16, p. 35). However, the vertical root temperature gradient changed root/shoot biomass ratio slightly towards one (Fig. 16, p. 35; App.17). High root/shoot biomass ratios are usually known with unfavorable root temperatures (sub- or supra-optimal, e.g. Engels, 1994; Davidson, 1969; in this study: 5°C uniform root temperature, data not shown) or at nutrient deficiency (Marschner *et al.*, 1996; Mattsson *et al.*, 1991). Increase in root/shoot biomass ratio due to nutrient deficiency could be excluded in the present experiments. Nutrient supply was sufficient at all root temperature treatments (including vertical root temperature gradient) due to continuous supply with nutrient solution of a concentration which is not limiting growth (cf. Chap. 2.3.1). Furthermore, sufficient supply was indicated by nutrient concentrations in shoots exceeding threshold deficiency values (e.g. 0.2 % Mg g^{-1} dry weight; Reinbott & Blevins, 1994; App. 19) and corresponding to values usual for vigorous plants (e.g. 0.05-0.06 g N g^{-1} dry weight; Lee & Drew, 1986; Fig. 31b, p. 50). It seemed unlikely that temperatures occurring along the gradient had to be regarded as unfavorable for growth. The temperatures were within the optimum range known for barley plants and highest amount of shoot as well as root dry weight was reached compared to uniform root temperature treatments examined in this study. Disparities in root tissue density (App. 7) could also not be responsible for higher root/shoot biomass ratio at vertical root temperature gradient compared to plants grown at 10°C, 15°C and 20°C uniform root temperature, since no difference in root tissue density occurred between plants grown at vertical root temperature gradient and plants grown at uniform root temperatures. However, the strongly accelerated aboveground plant development at 20-10°C RTG compared to uniform root temperatures described in Chap. 4.1.2 might cause the need of an enlarged root system to meet the demand for nutrients of the fast growing shoot.

4.1.4 Changes in carbon and nitrogen partitioning within the plant

Carbon and nitrogen are two of the most important elements in plant metabolism. Carbon is essential concerning energy transformation within the plant and production of plant tissue components. Nitrogen is an indispensable constituent of numerous organic compounds such as amino acids, proteins and nucleic acids as well as compounds of secondary plant metabolism (e.g. alkaloids) and may also be involved in plant signaling. According to Thornley's equilibrium model (1972), in the current study stable C/N ratios in both plant parts could be expected independent of root temperature treatment, since this model assumes that root and shoot depend on provision of each other; i.e. root growth depends on C from the shoot and its own acquisition of N. Therefore, a larger root system needs a larger shoot providing sufficient C for further root growth. A larger shoot is reached by increasing N supply from the roots (cf. Farrar & Jones, 2000; Marschner *et al.*, 1996).
However, in this study C/N ratios of shoots were equal at all root temperature treatments, due to a strong positive linear correlation between shoot dry weight and C as well as N content (R^2 = ~0.94-1.00, Fig. 27a, p. 46; Fig. 30a, p.49). The correlation between root dry weight and C as well as N contents was less significant compared to shoots (e.g. R^2 = 0.62 at 20-10°C RTG; Fig. 27b, p. 46; Fig. 30b, p.49). This indicated that C and N allocation in the plants was predominated by shoot demand independent of root temperature, resulting in C_{root}/C_{shoot} and N_{root}/N_{shoot} ratios below one.

4.1.4.1 Carbon allocation

Varying C allocation in plants grown at 10°C RT compared to 15°C RT, 20°C RT and 20-10°C RTG (Fig. 28a, p. 47) might be due to less restrictions in C allocation to roots and C assimilation than in root growth and respiration at low root temperatures (Engels, 1994). As respiration at low root temperatures decreases with a Q_{10} of 2 (Atkin *et al.*, 2000; Farrar, 1988; Lambers, 1985), accumulation of C allocated to the roots might be enhanced at the 10°C root temperature treatment (Equiza *et al.*, 2001; Deane-Drummond *et al.*, 1980). A C_{root}/C_{shoot} ratio nearly twice as high at 10°C RT compared to the other root temperature treatments examined in the present study supports this hypothesis. However, further research on contents of soluble carbohydrates has to be conducted to validate this assumption. Possible interactions between barley plants and microorganisms influencing C allocation to roots could not be completely excluded in the present study, although the experimental setup was

designed to guarantee equal constant conditions at all root temperature treatments. Nevertheless, preliminary studies on microorganism behavior at different root temperatures conducted in the present study revealed changed species richness of microorganisms with root temperature (personal communication Fang & Liebich).

Conformities in C partitioning in plants grown at 15°C, 20°C RT and 20-10°C RTG might be explained according to observations by Sowinski et al. (1998). They observed delayed root growth in the unfavorable subsoil temperatures for chilling-tolerant genotypes of *Zea mays*. Consequently, sink function (e.g. respiration) and thus assimilate translocation is not restricted by the lower subsoil temperatures occurring at a vertical temperature gradient in soil. Root growth at 20-10°C RTG was mainly restricted to soil temperatures of 20°C and about 15°C. Thus, the same total rate of respiration and therefore the same assimilate translocation pattern might be assumed at the vertical root temperature gradient as at 15°C and 20°C RT.

In agreement with results obtained with soybean (*Glycine max;* Rufty *et al.*, 1981) and corn (*Zea mays*) as well as wheat (*Triticum aestivum*; Engels, 1994), C concentrations in roots of barley decreased with increasing root temperature. Lowest C concentration was reached in roots of plants grown at 20-10°C RTG (Fig. 28b, p.47) due to reduced C concentration in roots of 0-5 cm depth (Fig. 29, p. 48). This might be explained by C use close to C import into roots at this depth (Loveys *et al.*, 2002), possibly due to especially high root growth and respiration or microorganism activity (cf. above) in this depth at 20-10°C RTG.

4.1.4.2 Nitrogen allocation

Plants grown at 10°C RT showed a higher N_{root}/N_{shoot} ratio than plants grown at higher uniform root temperatures and at vertical root temperature gradient (Fig 31a, p. 50). A special low N_{root}/N_{shoot} ratio was reached at 20-10°C RTG, but might result from experimental artifact, because in a repetition experiment, this ratio was similar to the one obtained at 15°C and 20°C RT (App. 18). The enhanced N allocation to the shoot at higher uniform root temperatures (i.e. 15°C and 20°C) as well as at 20-10°C RTG might result either from higher relative growth rate of the shoot in these plants compared to plants grown at 10°C RT (Mattsson *et al.*, 1991) indicated by accelerated plant development (cf. Chap. 4.1.2) or by altered localization of nitrate reductase activity (NRA). The fraction of NRA decreases in roots and increases in shoots with increasing root temperature (Macduff & Trim, 1986).

Therefore the hypothesis can be formulated, that the location of N assimilation in barley plants might have changed depending on root temperature.

The enhanced allocation of N to the shoot at 15°C and 20°C uniform root temperature and at 20-10°C RTG compared to allocation at 10°C RT resulted in quite similar N contents in plant roots of all research temperatures (Tab. 5, p. 49). Similar results were obtained by Gavito et al. (2001) comparing N contents in roots of winter wheat grown at 10°C and 15°C RT. Similar N contents in roots independent of root temperature treatment and root dry weight might indicate the persistence of a constant N pool in roots sufficient to meet the needs of the roots, whereas the N surplus was transported to the shoot for leaf growth and leaf initiation (Rufty *et al.*, 1981). Indeed it seemed likely, that the resulting extremely low N concentration in roots of plants grown at 20-10°C RTG marked the concentration sufficient for maintaining root growth, because it was in agreement with N data occurring in roots of gramineae under field conditions (0.01-0.015 g g^{-1} dry weight; Bahn *et al.*, 2006). In contrast, the higher N concentrations in roots of plants grown at uniform root temperature treatments (0.035-0.04 g g^{-1} dry weight) corresponded with N concentrations in roots of barley plants examined in hydroponics (Lee & Drew, 1986). These differences in root N concentration between plants grown at vertical root temperature gradient and uniform root temperatures might be due to differences either in N metabolism (cf. Chap. 4.1.4.3) or in long-range transport due to possible changes in internal root structure (cf. Chap. 4.2.2). Research at root tissue level and on xylem sap compounds would be necessary for explaining the mechanisms underlying the identified N concentration differences in more detail. Furthermore, comparing N concentrations between the different temperature treatments revealed strongly reduced N concentrations in each depth of the root system of plants grown at the vertical root temperature gradient compared to plants grown at uniform root temperatures (~0.01-0.025 vs. ~0.03-0.05 g g^{-1} dry weight, Fig. 32, p. 51). In general, the reduced N concentrations in all depths at 20-10°C RTG resulted from highest dilution effect due to highest root dry weight and highest N allocation to the shoot. These results support the hypothesis of accelerated aboveground plant development at 20-10°C RTG due to enhanced availability of growth determining compounds (e.g. N) formulated in Chap. 4.1.2.

4.1.4.3 Differences in N metabolism

As already mentioned before (cf. Chap. 4.1.4.2) differences in N metabolism might be responsible for differences in plant growth and development of plants grown at the vertical root temperature gradient compared to plants grown at uniform root temperatures. A closer look on the N status of plants grown at 20-10°C RTG and 15°C RT seems to support this hypothesis:

Nitrate reduction and assimilation in barley mainly occurs in the shoot at NO_3 concentrations > 1mM in nutrient solution (Mattsson *et al.*, 1988; Lewis *et al.*, 1982a; Sutherland *et al.*, 1985; Andrews, 1986), explaining the dominating N allocation to shoots at all root temperature treatments examined in the present study (cf. Chap. 4.1.4.2). Accumulation of metabolically inactive NO_3^- may occur in these plants due to NO_3^- uptake exceeding reduction capacity of the plant (Barneix *et al.*, 1984) described by higher NO_3^--N/total N fraction in roots than in shoots independent of root temperature (Engels, 1994). Agrell et al. (1997) showed storage effects in barley containing about 40-50 % $^{15}NO_3$-N/total ^{15}N in roots compared to about 15-20 % in shoots. In the current study, results obtained with unlabeled N for plants grown at 20-10°C RTG and 15°C RT were in agreement with these data (Fig. 34, p. 52).

In addition, the total free amino acid concentration in shoots of plants grown at 20-10°C RTG was twice as high as in plants grown at 15°C RT, while total free amino acid concentration in roots was similarly low (~ 0.05 % of fresh weight) at both root temperature treatments. The enhanced concentration of free amino acids in shoots at 20-10°C RTG might explain the high shoot dry weight production occurring at this root temperature treatment: High free amino acid concentrations in shoots indicate a high ability to initiate new leaves (Rufty *et al.*, 1981), since most amino acids will be used locally during vegetative growth (Imsande & Touraine, 1994). Glutamate and aspartate are important molecules in N transport and signaling within the plant (Aslam *et al.*, 2001; Vidmar *et al.*, 2000). Both free amino acids showed similar concentrations in shoots at both temperature treatments (Fig. 36, p. 53). This result in addition to equal concentrations of total free amino acids in roots might point to a quite stable amino-N pool cycling through the plant (Touraine *et al.*, 1994).

Protein concentrations differed in roots of plants grown at 15°C RT and 20-10°C RTG (Fig. 38, p. 54) and might be caused by varying lipid-protein interactions in the plasma membrane (Caldwell & Whitman, 1987). Dynamic of membrane proteins occur over a range of 12°C to 32°C and can be accounted for complex temperature dependence of the barley root

plasma membrane ATPase (Caldwell, 1987). Differences in protein concentrations between root depths of a single plant might indicate differences in activity. High protein concentration in roots of plants grown at vertical root temperature gradient in 5-10 cm depth compared to 0-5 cm depth corresponded with high root production within 5-10 cm depth in this temperature treatment.

It is known, that nitrate reductase activity in shoots of barley has a diurnal rhythm (Lewis *et al.*, 1982b; Sutherland *et al.*, 1985; Matt *et al.*, 2001b). Furthermore, phasing of the nitrate reductase activity is possibly influenced by N-nutrition (Matt *et al.*, 2001a) and day length (Matt et al., 1998). However, harvest of each root temperature treatment occurred at same time of the day and N-nutrition as well as day length was constant at all treatments. Therefore, data of proteins, free amino acids and nitrate are comparable in this study. Nevertheless, these results have to be regarded cautiously due to small sample size (n = 2-4), especially "shoot" data as they are actually results of single leaf analyses.

4.2 Vertical temperature gradients in soil cause changes in plant morphology

Plants do not only respond to root temperature on entire plant level by varying development and growth as well as C and N allocation. Structural and morphological responses in different plant parts are also evident.

4.2.1 Changed leaf structure

In contrast to leaf area of barley plants grown at different root temperatures and harvested at the same development stage (Sharratt, 1991), no difference in leaf area of plants of the same age revealed between the root temperature treatments examined in the present study. Specific leaf area (cm^2 leaf area g^{-1} leaf weight) on a fresh weight basis was also equal at all root temperature treatments (App. 10). This indicated no difference in leaf 'thickness' (Ryser & Lambers, 1995). When leaf area was related to leaf dry weight, a lower value was reached by plants grown at the vertical root temperature treatment compared to plants grown at uniform root temperatures (Fig. 14b, p. 34). This pointed to increased tissue density due to e.g. increased cell wall thickness or less expansion of mature cells (Lu & Neumann, 1999) in leaves of plants grown at 20-10°C RTG. This result was contrasting to the literature, because

fast growing plants generally show low tissue density to reduce their dry weight costs while maintaining high photosynthetic rate per unit leaf area (Ryser & Lambers, 1995). As plants grown at 20-10°C RTG showed fastest development and growth, similar results as stated in the literature would be expected. However, results obtained in this study should be interpreted cautiously, because results represent the properties of a particular leaf of the plants examined at the different root temperature treatments.

4.2.2 Root system plasticity

Exploring the soil by roots is essential for plants to acquire nutrients and water. Surviving at various soil conditions is enabled by root system plasticity. Depending on soil condition the root system of plants is developed in a special way (Bengough *et al.*, 2004; Hutchings & John, 2004; Briggs, 1978).

Barley plants used in this study developed a dichotomous root system typical for annual plants sufficiently supplied with nutrients (Fitter *et al.*, 1991; Dunbabin *et al.*, 2004). In general, the root system of *Hordeum vulgare* var. Barke is determined by 5-7 seminal roots mainly branching in 1st and 2nd order lateral roots. Additionally, some adventitious roots (crown-roots) may develop (Fig. 48). Commonly, these roots prefer the uppermost soil layers, are thicker and less branched than seminal roots but also take part in nutrient uptake (Briggs, 1978).

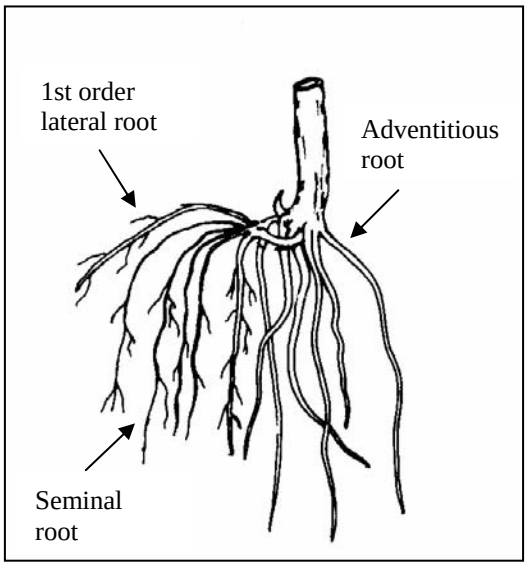

Fig. 48: Rooting pattern of *Hordeum vulgare* var. Vega (30 days) similar to *Hordeum vulgare* cv. Barke (after (Briggs, 1978).

4.2.2.1 Root length

In the literature, alterations of the root system are mainly determined by root length measurements (Kaspar & Bland, 1992). Findings of this study confirmed that root length is more sensitive to soil temperature than root dry weight as postulated by Kaspar & Bland (1992) and Bowen (1991). Root dry weight is rather a function of soil temperature and time than exclusively of soil temperature (c.f. Chap. 4.1.3 and 4.4). In contrast, the length of the entire root system is directly influenced by soil temperature due to its impacts on root diameter composition (i.e. fractions of different root diameters on entire root system) and the underlying variables root branching and root elongation (Kaspar & Bland, 1992).

Total root length significantly increases with root temperature (Cumbus & Nye, 1982; Abbas Al-Ani & Hay, 1983). Results obtained in the current study are in agreement with this finding. Plants grown at 20°C RT reached about 3.5 times as much root length compared to plants grown at 10°C. No significant difference occurred between total root length of plants grown at 10°C and 15°C RT. Supporting this result, no significant difference in root length of winter wheat grown at about 10°C and 15°C root temperature for 4 weeks was reported by Gavito et al. (2001). Remarkably, plants grown at 20°C RT and 20-10°C RTG also reached similar total root length, although root dry weight at 20-10°C RTG was about 77 % higher compared to root dry weight at 20°C RT (Fig. 21, p. 39). Therefore, equal total root lengths have to refer to differences in root morphology. The difference in root morphology between plants grown at 20°C RT and 20-10°C RTG did not only apply to general differences in root architecture, but also to variation in shape of the entire root system. This was indicated by differing root length distribution with depth between both treatments. While the fraction on total root length slightly decreased with increasing depth at 20°C RT, the highest root length fraction was located in 5-10 cm depth when plants were grown at 20-10°C RTG (App. 3).

Root diameter composition

Changes in root tissue density could be excluded as significant cause for root lengths changes in this study (App. 7). Therefore, root diameter measurements were chosen for obtaining information about the impact of root temperature on root system plasticity, since it was difficult to directly analyze branching and individual root elongation in the plant pot experiments (cf. Chap. 2.3). Roots of higher order are always thinner than roots they originate from, but diameter of roots within a root order does not significantly differ with temperature

(Miyasaka & Grunes, 1990; Abbas Al-Ani & Hay, 1983). Therefore, the average root diameter (Macduff *et al.*, 1986) and the root diameter composition of the entire root system are usually related to branching intensity.

Regarding the root diameter composition of the entire root system, higher fraction of thin roots (< 1.0 mm in diameter) was measured at 20°C RT compared to the other temperature treatments (Fig. 23, p. 41). Thin roots are usually lighter than thick roots (Macduff *et al.*, 1986). This explains why plants grown at 20°C RT could reach the same root length as plants grown at 20-10°C RTG with less root dry weight. No difference in fractions of single root diameters occurred between plants grown at 15°C RT and 20-10°C RTG. It could be hypothesized, that this was due to the mean temperature of the vertical temperature gradient (15°C). However, it is more likely that these similarities occurred due to restricted branching intensity (McMichael & Quisenberry, 1993; Schwartz *et al.*, 1987; Brouwer & Hoogland, 1964) and root elongation (Abbas Al-Ani & Hay, 1983; Stone & Taylor, 1983; Ching & Barber, 1979) at lower soil temperatures in the deeper substrate layers of the 20-10°C RTG treatment. At more favorable subsoil temperatures, plants grown at vertical root temperature gradient might have developed a more similar root diameter composition for the entire root system to plants grown at 20°C RT.

Despite of same fractions of root diameters generating the entire root systems at 15°C RT and 20-10°C RTG, their distribution with depth differed. Remarkably, at 20-10°C RTG root diameter composition in 0-5 cm depth was the same as at 20°C RT in this depth (Fig. 24, p. 42). In contrast, the diameter composition in 5-10 cm depth (mean root temperature about 16°C) corresponded to plants grown at 15°C RT in 0-5 cm depth and not in 5-10 cm depth as possibly expected. That means a high fraction (~ 40 %) of thin roots (< 1.0 mm diameter) as well as of thick roots (~ 2.5 %; 2.0-3.0 mm diameter) compared to the uniform root temperature treatments was present in 5-10 cm depth, when plants were grown at 20-10°C RTG. This high fraction of thin roots explained why similar root length could have been reached as in 0-5 cm depth (App. 6), although root dry weight was about 70 % less. The high fraction of thin roots might occur due to stimulated lateral root production at root temperature change from 20°C to about 15°C (Tab. 3, p. 44) provided that results obtained for temporal root temperature changes can be transferred to spatial root temperature heterogeneity. The higher fraction of thick roots, i.e. the deeper rooting of thick roots might indicate a different uptake strategy developed when barley plants grew at vertical soil temperature gradients compared to plants grown at uniform root temperatures. For instance,

they might enhance the long-range transport of nutrients into the shoot supporting the accelerated aboveground plant development and growth at 20-10°C RTG.

Root branching and elongation

Brouwer (1962) stated that root temperature effect on root branching is more distinct than on root elongation. It is known, that root elongation decreased with root temperature (Abbas Al-Ani & Hay, 1983; Stone & Taylor, 1983). Similar effects were shown in rhizotrone experiments conducted in the present study, although no difference between root elongation at 15°C and 20°C could be detected.

Furthermore, these experiments demonstrated that lateral root production is more affected in its dynamics than in quantity. In the current study, the difference in total number of lateral roots was small between root temperature treatments at harvest time. This result is contrasting to findings in the literature reporting increased number of lateral roots with increasing root temperature (Cumbus & Nye, 1982; McMichael & Quisenberry, 1993; Schwartz *et al.*, 1987; Brouwer & Hoogland, 1964). Furthermore, temporal changes in temperature from 20°C to 15°C RT strongly stimulated lateral root production per time (Tab. 3, p. 44) as well as branching intensity per seminal root (Fig. 25, p. 43) compared to constant root temperatures or changed temperature into the opposite direction (10°C to 15°C).

Little is known about root branching mechanisms and changes may be caused by varying primordia initiation or lateral root emergence from primordia (Malamy, 2005). Differences in auxin movement to the roots essential for development of early stage primordia (Laskowski *et al.*, 1995) and lateral root emergence - at least in Arabidopsis seedlings (Malamy, 2005) - may be responsible for those variation. However, lateral root emergence in older seedlings seems independent of shoot-derived auxin suggesting that the root system may produce auxin itself and that different programs are involved in lateral root production depending on plant age (Bhalerao *et al.*, 2002).

Another mechanism explaining differences in branching intensity is glutamate sensing by roots (Filleur *et al.*, 2005). Primary root growth of Arabidopsis is inhibited by glutamate, whereas growth of lateral roots seems to be more stimulated at first - perhaps due to inhibited primary root growth -, but showing the same response as primary roots later in their development. However, root tips respond only to the immediate external presence of glutamate and not to glutamate supplied to other parts of the root by triggering a reduction in

rate of cell production and/or cell expansion (Filleur *et al.*, 2005). This effect has to be rejected explaining stimulated branching at temporal root temperature changes from 20°C to 15°C RT observed in this study, since no difference in elongation rate of individual roots as well as in total root length occurred when compared with plants grown at constant root temperature of 20°C. Furthermore, external glutamate mainly had to be recaptured by the plants from exudation, because extracellular organic matter as glutamate source was scarce due to conducting experiments in sand. Additionally, fertilizer input was high causing that total amino-N fraction on total N uptake by roots was < 30 % (Jones & Darrah, 1994). It is possible that exudation of plants was changed by temperature but this was not studied here.

NO_3^- was homogeneously distributed and its concentration was not limiting to plant growth in the current study. Therefore, external NO_3^- signaling was excluded as reason for enhanced intensity of lateral root production (Filleur *et al.*, 2005; Drew, 1975) at temporal root temperature change from 20°C to 15°C. According to Zhang et al. (1999) NO_3^- signaling also acts systemically regulating the allocation of resources between shoots and roots and thus determining root growth. High levels of NO_3^- in shoots are associated with reduced root growth. Furthermore, NO_3^- accumulation in the shoot might also reduce root branching by inhibiting auxin biosynthesis or auxin transport to the roots (Forde 2002). As NO_3^- levels of plants were not determined in the experiment with temporally changing temperatures, lower NO_3^- levels could not be excluded in shoots of plants exposed to temporal temperature change from 20°C to 15°C compared to constant root temperatures. However, presuming that temporal temperature change might cause similar metabolic effects as spatially changing temperature from 20°C to about 15°C, it was possible to exclude internal NO_3^- signaling as reason for enhanced root branching (Zhang et al., 1999; Zhang & Forde, 2000), since NO_3^- concentration in shoots was equally low at 15°C RT and 20-10°C RTG.

4.2.2.2 Rooting depth

Root temperature also influenced rooting depth by causing differences in root architecture. Deepest rooting was reached by plants grown at 20°C RT in pot experiments (depth of the plant pot was not limiting). Increased root elongation at 20°C RT compared to 10°C RT and the more pronounced investment into root proliferation than into lateral root initiation might be responsible for this as well as for similar absolute root lengths and root diameter compositions in each depth (0-15 cm) at 20°C RT.

Comparable rooting depths of plants grown at 10°C, 15°C RT and 20-10°C RTG may be explained by the following concept: Deep rooting at 10°C RT is restricted to some extend by reduced root elongation (Abbas Al-Ani & Hay, 1983; Stone & Taylor, 1983; Ching & Barber, 1979; cf. Chap. 4.2.2.1). At 15°C RT competition for carbohydrates (Stone & Taylor, 1983) and nutrients between seminal and adventitious roots (roots 2.0-3.0 mm diameter) in the uppermost substrate layers might restrict deeper rooting, since no difference in root elongation between 15°C and 20°C RT was reported. Deep rooting of plants grown at the 20-10°C RTG seemed to be limited by lower temperatures (< 15°C) in 10-20 cm depth. This is in agreement with results obtained by (Sowinski *et al.*, 1998) as well as by results obtained for plants grown at 15-3.5°C RTG (App. 20). Further explanations for this restricted deep rooting are on the one hand stimulated branching density (lateral roots per seminal root; cf. Chap. 4.2.2.1) and competition for carbohydrates in the uppermost substrate layers as already explained for plants grown at 15°C RT. On the other hand, the lower root temperatures may eventually also influence inclination of roots and therefore restrict growth to the more favorable temperatures of the uppermost 10 cm in the planting pot (Fortin & Poff, 1990; Kaspar *et al.*, 1981). However, influence of root temperature on growth angle of roots is controversially discussed (Kaspar & Bland, 1992). Nevertheless, as plants grown at 20-10°C RTG were not adapted to lower root temperatures when reaching them in the subsoil, root proliferation might be more restricted than with plants already adapted the entire root system to lower root temperatures (e.g. plants in the 10°C RT treatment).

4.3 Vertical temperature gradients in soil influence nutrient uptake and translocation in plants

As discussed in the previous chapters vertical temperature gradients in soil cause specific changes in root morphology. In the following chapters it will be discussed, whether these changes may influence nutrient uptake and translocation and therefore may be responsible for enhanced plant growth.

4.3.1 Direct effects of root temperature on nutrient uptake and translocation

Before examining effects of root morphology adapted to root temperature on nutrient uptake and translocation, direct root temperature effects on these processes will be examined. This was important, since otherwise it would not be possible to distinguish between direct or

indirect (i.e. changed root morphology) effects of root temperature on nutrient uptake and translocation.

4.3.1.1 Nitrogen

Numerous short-term experiments have been conducted examining the influence of root temperature on nutrient uptake and translocation. Generally, sub-optimal root temperatures reduce nutrient uptake as well as translocation to the shoot. This was shown by results obtained with excised roots (e.g. Bravo & Uribe, 1981) as well as with intact plants (Engels *et al.*, 1992; White *et al.*, 1987). Nevertheless, the sensitivity of uptake and translocation to root temperature may change with type of nutrient (valance and charge) and depend on processes and mechanisms involved in uptake.

In the present study, nutrient uptake kinetic was examined in the hydroponics experiment. Results of this experiment were in agreement with findings of short-term experiments on nutrient uptake stated above. The short-term exposure (0.5-48 h) of barley plants to labeled nutrient solution showed significantly lower ^{15}N/total N fraction in shoots of plants labeled at 10°C root temperature compared to plants labeled at 15°C and 20°C root temperature (Fig. 42, p. 64). This difference resulted from a decrease in ^{15}N uptake (Tab. 11, p. 64) with a Q_{10}-value of about 2 after 3 h of labeling and slightly increasing with labeling time. As N uptake is an active process, this Q_{10}-value was in agreement with results concerning active ion absorption processes in general ($Q_{10} \geq 2$; Schwartz *et al.*, 1987). In the present study, nitrogen uptake occurred via symport (probably $2H^+$:$1NO_3^-$; Glass *et al.*, 1992) and constitutive low affinity transporters (LATS; Crawford & Glass, 1998), because NO_3 concentration in the nutrient solution was sufficient for vigorously growing plants (> 2mM; Vidmar *et al.*, 2000).

It is hypothesized, that reduced ^{15}N uptake at 10°C RT occurred due to variation in the function or composition of LATS. Decreased rates of metabolic energy production at low temperatures and reduced availability of energy required for active uptake processes (Rufty *et al.*, 1981; Lee *et al.*, 2004) as well as reduced mobility of membrane phospholipids (Bravo & Uribe, 1981; Caldwell & Whitman, 1987) are possible explanations. The nutrient demand of the shoot was equal at all temperature treatments due to same shoot dry weights (App. 14). Therefore, regulation of uptake by signaling effects from the shoot to the root via e.g. amino acids (Touraine *et al.*, 1994) could be excluded, especially as LATS are less sensitive to amino acid signaling than HATS (high affinity transporters, dominating uptake at low external N concentrations; Aslam *et al.*, 2001). Nitrate reductase was also excluded being the

limiting factor for nitrogen uptake at 10°C RT (Beevers & Hagemann, 1980), because NO_3^- uptake mostly exceeds reduction capacity of the plant (Barneix *et al.*, 1984). This was indicated by increasing $^{15}N_{root}/^{15}N_{shoot}$ ratio with time (Tab. 10, p. 64) and high fraction of NO_3-N in roots compared to shoots independent of root temperature (Fig. 34, p. 52), since translocation of NO_3^- to the roots is not possible via phloem (Allen & Raven, 1987; Imsande & Touraine, 1994). Furthermore, differences in nutrient absorption area (cf. Chap. 4.3.2; Engels, 1993) was excluded due to same root dry weight and structure caused by equal pre-treatment conditions.

Although $^{15}N_{root}/^{15}N_{shoot}$ ratio increased with time (0.5-9 h) nitrogen allocation (^{15}N) to roots and shoot did not differ between the three labeling temperatures. Constant ^{15}N partitioning between roots and shoots at all temperature treatments indicated that N translocation did not respond sensitive to root temperature within the chosen timeframe.

4.3.1.2 Magnesium

In general, ^{25}Mg uptake and translocation was delayed compared to ^{15}N, but ^{25}Mg/total Mg fraction in shoots was also decreased at low (10°C) root temperature (Fig. 45, p. 68). In contrast to ^{15}N, this was not only due to reduced uptake (Tab. 12, p. 68) but also to reduced allocation to the shoot (Tab. 13, p. 69). Interestingly, plants labeled at 15°C RT also showed reduced uptake and translocation revealing with increase in time, whereas ^{15}N uptake at 15°C performed similar to that at 20°C RT. Comparable effects as seen for ^{25}Mg were identified, when plants were labeled with ^{44}Ca at 10°C and 20°C RT for 24 h (Chap. 3.5.2.1).

Mg uptake is assumed to occur passively via diffusion and apoplastic pathways across the root cortex (Kuhn *et al.*, 2000; Mengel *et al.*, 2001). Translocation occurs mainly from the apical regions of the root to the shoot, where the endodermis is not suberized. Calcium behaves quite similar as magnesium in uptake and translocation (Ferguson & Clarkson, 1976). Lower uptake and translocation of ^{25}Mg at lower root temperature has been attributed to higher viscosity of apoplastic fluids (Lang, 1974; Farrar, 1988). Results obtained for Ca uptake after 24 h of labeling may be explained by the same effect. Transpiration differences (Nkansah & Ito, 1995) could be excluded, since the transpiration effect on Mg and Ca is weak (Marschner, 1995).

The same experiment was repeated in plant pots and sand (cf. Chap. 2.2.3) with 9 h of labeling for validating the results obtained in hydroponics under largely natural conditions. Furthermore, the impact of the 20-10°C RTG on nutrient uptake could be shown in this experiment. The slightly reduced ^{15}N fraction in shoots of plants labeled in plant pots at 15°C root temperature compared to results obtained in the hydroponics experiment (Fig. 43a, p. 66) could be interpreted as stress reaction. This reaction occurred due to sudden exposition of plant roots to lower temperatures, since plants had to be immediately labeled when transferred from room temperature to experimental temperature without adaptation time. Plants labeled at 10°C seemed to be neither adapted to this particularly low root temperature in hydroponics nor in plant pots, because no difference in ^{15}N fraction in shoots occurred between both treatments. It was suggested, that plants labeled at 20-10°C RTG were also slightly stressed, indicated by comparable values as shown by plants labeled at 15°C in sand. It was assumed, that without stress effect the ^{15}N fraction in shoots at 20-10°C RTG had to be in the range of those obtained for 15°C and 20°C RT in hydroponics. Regarding ^{25}Mg fractions, no stress reaction in plants labeled at 15°C RT was observed (Fig. 46a, p. 70) due to passive uptake and apoplastic transport through the root cortex. Therefore, potential stress reaction was also excluded for plants labeled at 20-10°C RTG in sand.

4.3.2 Nutrient uptake and translocation of plants morphologically adapted to root temperatures

At long-term exposure plants adapt to low root temperatures by increasing the relative size of their root system and therefore the potential absorption area (Touraine *et al.*, 1994; Engels, 1993) or by enhancing the capacity of ion uptake (Clarkson, 1976; White *et al.*, 1987). This is reached by increasing the number of ion transporters in the root plasma membrane (Clarkson, 1976; Siddiqi *et al.*, 1984) or by increasing nutrient translocation to the shoot (Clarkson, 1976). In general, it is assumed that shoot demand controls nutrient uptake rather than being controlled by it when plants are adapted to root temperature (Macduff & Hopper, 1986; Cumbus & Nye, 1982; Engels, 1993; Mattsson *et al.*, 1992). Depending on RGR of the shoot, inflow (defined as uptake rate per unit root length) may differ even when root/shoot biomass ratios were similar between the different root temperatures.

In the current study, plants germinated and grown at different root temperatures showed similar effects as shown for long-term exposure experiments mentioned above. Examining nutrient uptake and translocation of these plants revealed none of the temperature effects

appearing in short–term experiments with ^{15}N discussed above (Fig. 43a, p. 66). In contrast, results of ^{25}Mg/total Mg fractions in shoots were the same as in short-term experiments (Fig. 46a, p. 70).

4.3.2.1 Nitrogen

Hardly any difference was observed between ^{15}N fractions in shoots of plants morphologically adapted either to uniform root temperatures or to 20-10°C RTG. The straight relation of ^{15}N and ^{25}Mg content in shoots with shoot dry weight shown at all temperature treatments (Fig, 43b, p. 66; Fig. 46b, p. 70; App. 17) confirmed the thesis of nutrient uptake mainly determined by shoot demand (Reinbott & Blevins, 1994). The enhanced shoot demand at increasing root temperatures and especially at 20-10°C RTG seemed to be satisfied by increasing the absorption area, i.e. the root surface area (Fig. 18, p. 38). However, regarding root surface area/total plant dry weight (Fig. 20, p. 38) as variable determining potential nutrient uptake efficiency, roots of plants grown at 20-10°C RTG seemed to be more efficient in uptake per surface area compared to plants grown at uniform root temperatures. No significant difference was determined between uniform root temperature treatments, whereas plants grown at 20-10°C RTG showed a very low value (0.03-0.035 vs. ~ 0.017 $m^2\ g^{-1}$ dry weight). Root diameter and root tissue density are important traits concerning supply and storage properties involved in plant nutrition. As root tissue density did not differ between the root temperature treatments, it seemed likely, that potential higher uptake efficiency at 20-10°C RTG resulted either from the adapted root diameter composition or from changed root diameter distribution with depth. Especially since the root diameter composition in 5-10 cm depth is significantly different at 20-10°C RTG compared to uniform root temperatures. Complementary research on nutrient inflow and transport through the root cortex (e.g. localization of stable isotopes in roots) is needed to validate postulated higher efficiency of root systems at 20-10°C RTG (Kuhn *et al.*, 2000).

Taking all discussed aspects into account, it can be stated that direct root temperature effects on active nutrient uptake and translocation are overridden by effective, indirect root temperature effects concerning plant morphology. It was not possible to distinguish yet, whether adaptation of root morphology or of plant growth to the respective root temperatures is more responsible for equal ^{15}N fractions occurring in the shoots.

Plants grown at 20-10°C RTG contained more total N than plants grown at 20°C RT. Additionally, the ^{15}N content in shoots was highest at 20-10°C RTG. This indicated either enhanced translocation to the shoot or higher total uptake. Both processes might be responsible for the enhanced plant growth at 20-10°C RTG. However, due to missing ^{15}N data of the entire root no final validation of one of these statements was possible.

4.3.2.2 Magnesium

Similar fractions of ^{25}Mg on total Mg in shoots irrespective of morphological adaptation to the respective root temperature can be related to the passive uptake by diffusion. Since an equal gradient in Mg concentration between the external solution and the plant internal can be assumed, the amount of total ^{25}Mg taken up at time mainly depends on root biomass. Root biomass was the same at all temperature treatments in the short-term experiment. In the experiment with morphologically adapted plants root biomass was simultaneously adapted with shoot biomass to the respective root temperature. Therefore, the fraction of ^{25}Mg on total Mg in shoots was similar in both experiments. The slight temperature effects seen in the short-term experiment persisted, because mechanisms determining Mg uptake and translocation (differences in viscosity in the free space and in diffusion) were still working in the same way.

It can be stated, that with passive nutrient uptake direct temperature effects are more important for uptake and translocation than morphological changes caused by root temperature.

4.3.3 Nutrient uptake in dependence of root depth

When comparing nutrient uptake of plants labeled in different root depths (1 cm and 6 cm below surface, respectively), results followed the same pattern at both labeling treatments. It did not matter, if particular roots were excluded from uptake or if roots were morphologically adapted to the respective root temperature treatment (10°C, 15°C and 20°C uniform root temperature and 20-10°C vertical root temperature gradient; Fig. 43a, p. 66 & Fig. 44a, p. 67; Fig. 46a, p. 70 & Fig. 47a, p. 70).

These findings might indicate that it does not matter which type of roots is involved in N and Mg uptake. For N uptake this might be true, because N uptake in barley occurs along the

entire root and does not widely differ between root types (Krassovsky, 1926). However, Huang & Grunes (1992) observed for barley and Norway spruce (*Picea abies* L.) that Mg uptake is highest at root apex and decreases towards the base. Therefore, branching and location of root tips is more important for uptake of Mg. As no differences in ^{25}Mg/total Mg shoot between the both labeling treatments could be detected (Fig. 46a, p. 70; Fig. 47a, p. 70) it is hypothesized that most root tips were present in 6-20 cm depth, independent of root temperature treatment.

4.4 Plant age and development stage influence plant response to soil temperature

Plant response to soil temperature may change during aging and ontogeny (Miyasaka & Grunes, 1990; Huang & Grunes, 1992; Brouwer, 1964). Therefore, experimental results might be misleading when these aspects are not taken into account. The significance of taking plant age and development stage into account when interpreting responses to varying soil temperatures became evident in the present study, since both situations were simultaneously examined for the first time.

As discussed in Chap. 4.1.3, plant growth is accelerated by soil temperature, but depending on age or development stage at harvest time the impact of soil temperature on plant growth can be interpreted in different ways. This may explain confusion in describing root temperature effects on biomass allocation as well as on dry weight obtained at different root temperatures in the literature. However, it seems that plant species also determines the way of changing responses to root temperature during plant aging.

Beauchamp & Lathwell (1967) reported increasing dry weight with decreasing root temperature in shoots and roots of young corn compared at the same leaf stages. This is a very common finding stated by many authors (e.g. Beauchamp & Lathwell, 1967; Miyasaka & Grunes, 1990). It is assumed that plant development is relatively faster than plant growth at increasing root temperatures. Consequently, dry weight of plants grown at higher root temperatures is lower than of plants grown at lower temperatures when plants are compared at the same development stage due to the shorter time plants needed to reach the next development stage at increasing root temperatures compared to plants grown at lower root temperatures. In contrast, Cumbus & Nye (1982) found no difference in dry weights and root/shoot ratios of rape (*Brassica napus* cv. Emerald) grown at different root temperatures

and compared at the same leaf stage. On the other hand, Sharratt (1991) obtained decreasing root/shoot ratios with increasing root temperature for barley plants grown at 5°C, 10°C and 15°C RT and compared when awns emerged from the leaf sheath.

Similar results as by Sharratt (1991) were obtained in the present study for barley plants younger than 22 days. Root as well as shoot dry weight was higher when plants were grown at 20°C compared to 10°C RT (Fig. 39, p. 57), no matter if plants were compared at the same development stage or at the same age. Furthermore, plants grown at 20°C RT showed lower root/shoot ratios compared to plants grown at 10°C RT. Similar to results of Cumbus & Nye (1982), barley aged ≥ 22 days did not differ in root/shoot ratio when compared either at the same development stage (Fig. 40, p. 58) or the same age. This was evident, although differences in biomass occurred when plants were compared at the same age (Fig. 16, p. 35). Power et al. (1970) reported a comparable effect of dry weight adaptation after exceeding a particular age or development stage with barley plants grown to maturity.

Beside equal biomass (shoot as well as root dry weights), total root length and surface area as well as their distribution with depth were similar, when barley plants grown at 10°C and 20°C RT and exceeding day 22 were compared at the same development stage. Additionally, C and N concentration was equal at the entire plant level as well as in root and shoot. Distribution of N concentration in roots differed with depth (Tab. 9, p. 61). Interestingly, the distribution pattern of N seemed to persist with plant age. Plants grown at 20°C RT showed the same distribution pattern at day 24 (compared with plants grown at 10°C RT at the same development stage) as at day 30 (compared with plants grown at 10°C RT at the same age). Therefore, N distribution patterns for each root temperature seemed to be committed independent of plant age and development stage at least when plants were older than 22 days. Furthermore, it is known that nitrogen uptake rates vary with plant age as well as with root temperature. However, the influence of root temperature on uptake rate may also change with plant age (Schwartz *et al.*, 1987). This effect is also shown for Mg uptake rate in wheat. In plants < 30 days the uptake rate increases with increasing root temperature, while it decreases with increasing root temperature when plants > 30 days (Huang & Grunes, 1992).

The same effect obtained for N distribution with depth was observed for root diameter composition. Fractions of individual root diameters at different depths varied with plant age and development stage. Therefore, differences in root diameter composition occurred between the root temperature treatments in any case. Furthermore, the pattern of root mass distribution

with depth seemed to be independent of plant age and development stage, since similarities in root mass fractions at different depth occurred either between plants of the same development stage (10°C RT vs. 20°C RT, Tab. 7) or of the same age (10°C RT vs. 15°C RT vs. 20-10°C RTG, App. 1a).

Therefore, one has to be aware that root temperature effects observed with plants may occur in changing amplitudes during the plant life cycle. Results obtained either for plants compared at the same age or at the same development stage can only represent a snapshot in plant responses to soil temperature.

5 The significance of vertical soil temperature gradients – conclusion & outlook

5.1 Conclusion

The experimental setup used in this study allowed the identification of plant responses caused exclusively by root temperature under controlled but relatively natural conditions. This study compared for the first time, effects caused by a vertical gradient in root temperature with results observed at uniform root temperatures. With this approach it was possible to assess the significance of previously published results obtained with uniform root temperatures against the background of fluctuating environmental conditions.

It was shown that barley plants clearly benefit from growth at vertical root temperature gradients in the vegetative stage and that they morphologically as well as physiologically differ from plants grown at uniform root temperatures. In detail, the following answers were obtained (cf. Chap. 1.4):

- Does a vertical gradient in soil temperature alter plant growth during the vegetative stage compared to plants grown at uniform soil temperatures?

  A vertical gradient in soil temperature accelerated plant development and growth.

- (a) Do changes in plant structure and/or function occur at vertical soil temperature gradients compared to uniform soil temperatures? (b) Are these changes responsible for possible differences in plant growth?

  (a) The root system of plants grown at the vertical root temperature gradient was characterized by shallow rooting and a high fraction of thicker roots (≥ 1.00 mm diameter). This corresponded to the response of plants grown at 15°C RT. However, in contrast to 15°C RT, plants grown at 20-10°C RTG did not reach highest fraction of total root length in 0-5 cm. They reached highest fraction of total root length in 5-10 cm depth, although less root dry weight was present in 5-10 cm compared to 0-5 cm depth at both root temperature treatments. This was explained by differences in fractions of individual root diameters within the respective depths and compared to plants grown at 15°C RT.

(b) No significant differences between 20-10°C RTG and 15°C RT occurred, when nutrient uptake and translocation were analyzed. However, in general it has to be stated that at active nutrient uptake processes direct root temperature effects, e.g. lower N uptake at 10°C RT compared to higher root temperatures, were overridden by adaptation of plant structure to the respective root temperature. This underlines the importance of structural traits (e.g. biomass allocation to the shoot, fractions of individual root diameters) for nutrient demand and supply. A higher concentration of most individual free amino acids was found in shoots at 20-10°C RTG and differences between protein concentrations in roots of plants grown at 20-10°C RTG versus 15°C RT occurred. This indicated differences in the dynamics of N metabolism of plants in the varying temperature treatments.

⇒ It was shown that a vertical gradient in root temperature influences plant structure and function in a different way than the respective uniform root temperature representing the average temperature of this gradient.

It was hypothesized, that plants at a vertical root temperature gradient grow faster than plants at uniform root temperatures due to a combination of structural and functional components making nutrient uptake, nutrient translocation and nutrient use more efficient.

- Do plant responses to soil temperature vary with plant age and development stage?

  The amplitude of root temperature effects on plant structure changed during plant aging.

5.2 Outlook

Results of the present study indicated that a vertical root temperature gradient caused variations in plant performance compared to uniform root temperatures, and the extent of these effects on plants was previously unknown. However, at the current stage of research mechanisms underlying these variations could not be explained in full detail. Further research is required on metabolic activities within the plant in response to root temperature gradients and should also take proteomic as well as genetic analyses into account. Additionally, signs of enhanced nutrient uptake efficiency of plants grown at a vertical root temperature gradient found in this study should be followed by examining nutrient transport across the root cortex at cell and tissue level.

In addition, findings of the present study suggest that recent predictions concerning plant development and biomass production in the field have underestimated these processes. When modeling plant development and biomass production, effects caused by root temperature were either neglected or data used were related to results obtained with uniform root temperatures in artificial systems such as hydroponics or plant pots in greenhouses (cf. Yan & Hunt, 1999; Kirby, 1995). Since the results of the present study were obtained only for plants in the vegetative stage, it would be essential to unravel whether fruiting bodies and crop yield may also benefit from growth at vertical root temperature gradients. Such follow up experiments would be best repeated with various plant species and different vertical soil temperature gradients simulating varying environmental conditions found under field conditions. With this additional knowledge it should be possible to improve predictions on growth and yield of plants and also to start thinking about implementing the beneficial effect of vertical root temperature gradients in greenhouse production.

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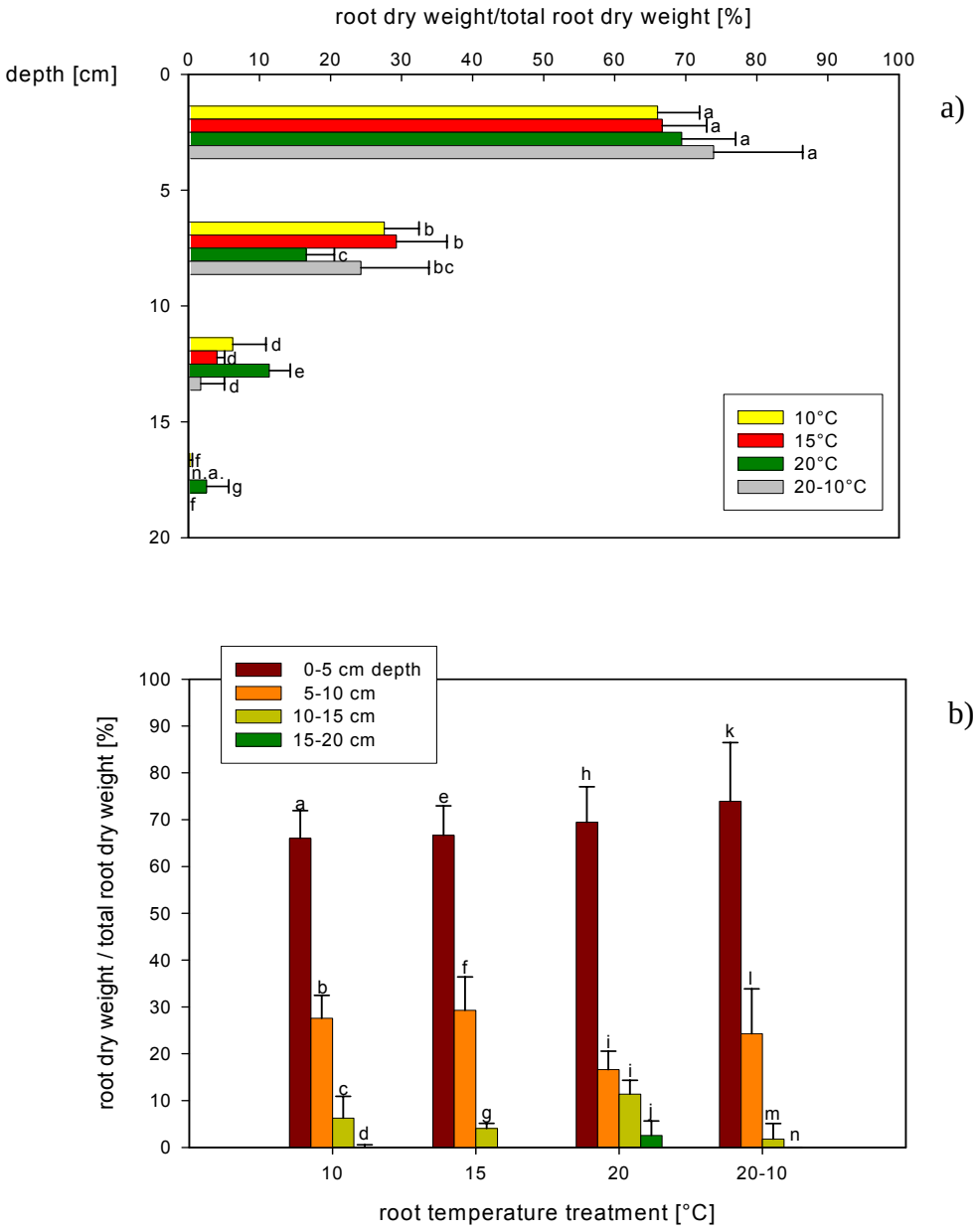
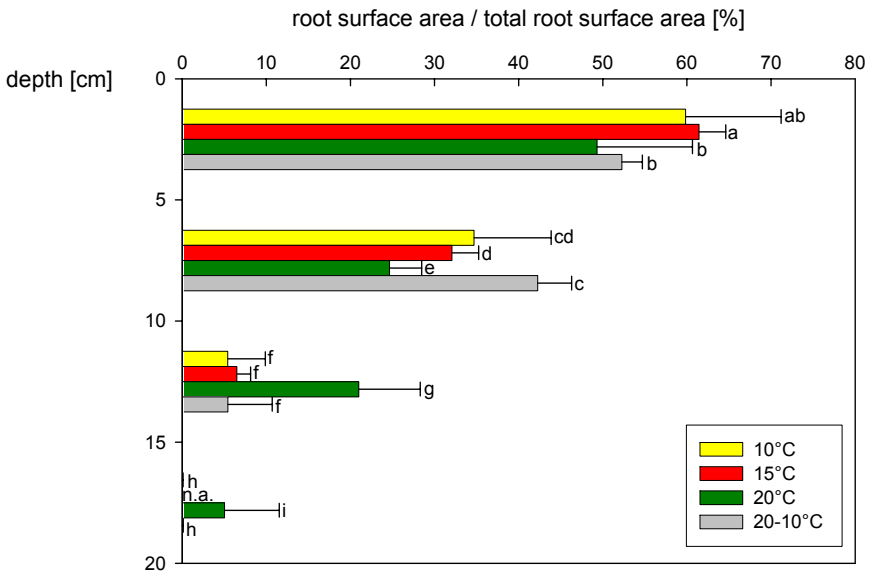
Appendix

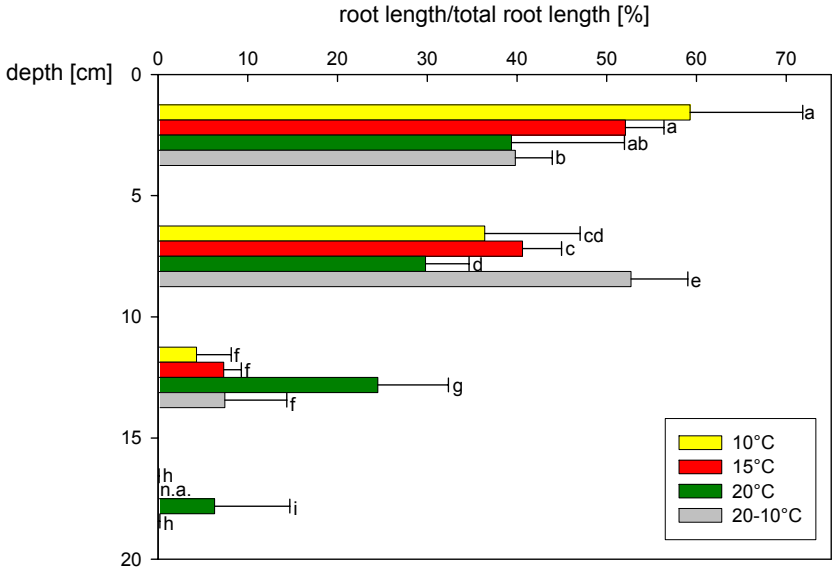
A Further results of plants grown in plant pots at different root temperature treatments



App. 1: Fraction of root dry matter at depth [% total root dry matter], a) compared at depth, b) compared at root temperature treatment. Mean and standard deviation are shown. Statistical analysis was done by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences, n.a. = not available.



App. 2: Fraction of root surface area at depth [% total root surface area]. Mean and standard deviation are shown. Statistical analysis was done by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences, n.a. = not available.



App. 3: Fraction of root length at depth [% total root length]. Mean and standard deviation are shown. Statistical analysis was done by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences, n.a. = not available.

Root mass [mg dry weight]

Root temperature treatment [°C]	Depth [cm]			
	0-5	5-10	10-15	15-20
10	58.04[a]	24.97[b]	5.58[c]	0.12[d]
15	96.11[a]	42.32[b]	5.75[c]	n.a.
20	148.53[a]	33.49[b]	22.68[c]	6.46[d]
20-10	646.99[a]	195.45[b]	16.46[c]	0.03[d]

App. 4: Root mass at depth compared within one root temperature treatment. Mean is shown. Statistical analysis was done by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences, n.a. = not available.

Root surface area [cm²]

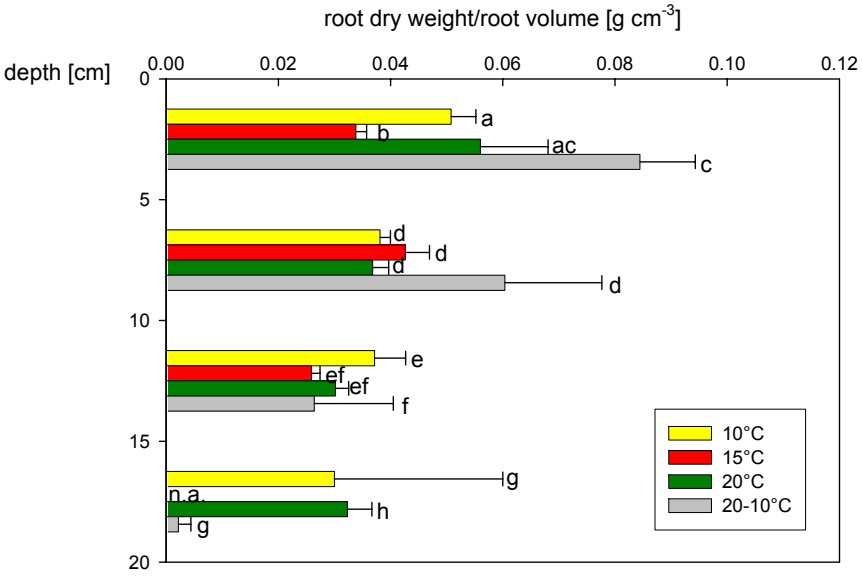
Root temperature treatment [°C]	Depth [cm]			
	0-5	5-10	10-15	15-20
10	61.93[a]	35.36[b]	6.49[c]	0.03[d]
15	97.95[a]	50.33[b]	10.16[c]	n.a.
20	137.72[a]	68.28[b]	60.31[b]	18.27[c]
20-10	228.83[a]	183.59[a]	24.36[b]	0.12[c]

App. 5: Root surface area at depth compared within one root temperature treatment Mean is shown. Statistical analysis was done by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences, n.a. = not available.

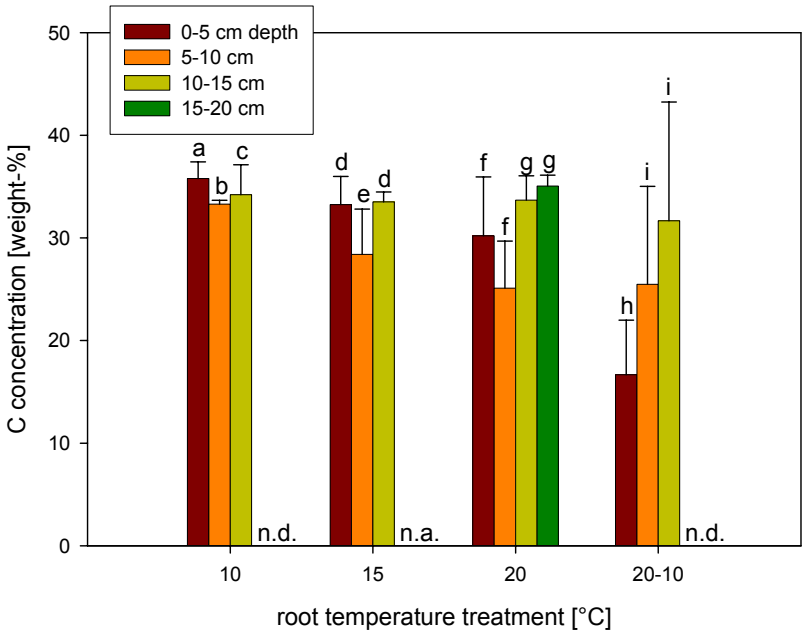
Root length [cm]

Root temperature treatment [°C]	Depth [cm]			
	0-5	5-10	10-15	15-20
10	259.23[a]	156.84[b]	22.57[c]	0.18[d]
15	271.25[a]	206.58[b]	37.11[c]	n.a.
20	541.01[a]	414.62[a]	364.39[a]	122.31[b]
20-10	587.19[a]	756.80[a]	119.89[b]	0.73[c]

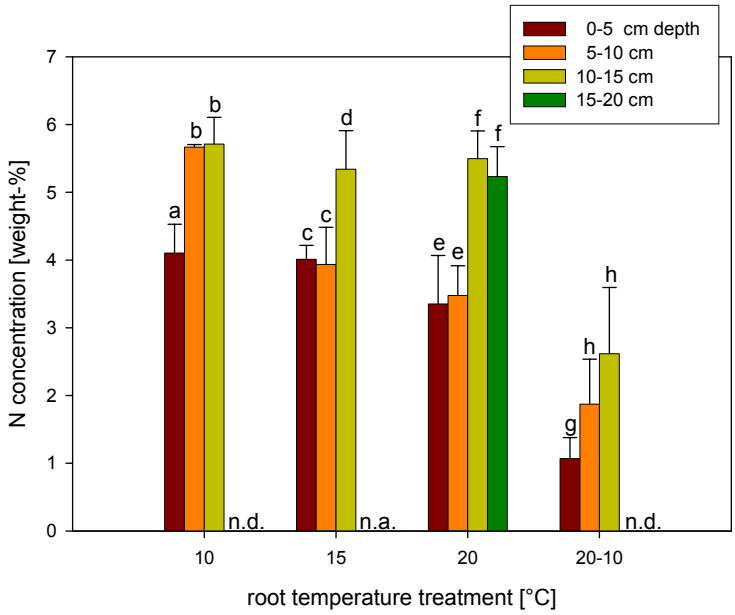
App. 6: Root length at depth compared within one root temperature treatment. Mean is shown. Statistical analysis was done by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences, n.a. = not available.



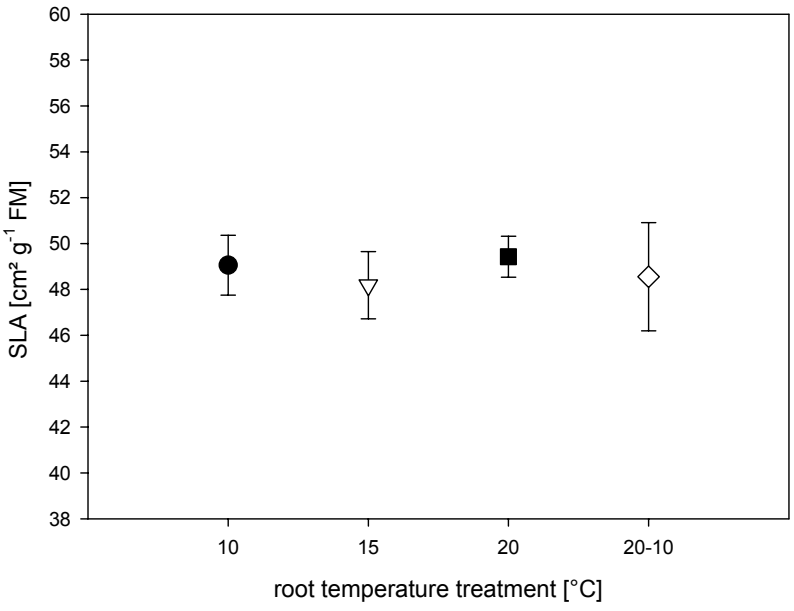
App. 7: Root tissue density [root dry matter/root volume] differentiated by depth. Mean and standard error of the mean are shown. Statistical analysis was done by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences; n.a. = not available.



App. 8: C concentration [% dry weight] at depth compared at root temperature treatment Mean and standard deviation are shown. Statistical analysis was done by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences; n.a. = not available, n.d. = not determined.



App. 9: N concentration [weight-%] at depth compared at root temperature treatment. Mean and standard deviation are shown. Statistical analysis was done by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences; n.a. = not available, n.d. = not determined.



App. 10: Specific leaf area (SLA) related to fresh mass. Mean and standard error of the mean are shown. Statistical analysis was done by one way ANOVA and t-test; $p < 0.05$; $n = 8$. There is no statistically significant difference between the root temperature treatments.

B Further data concerning N metabolism

Root temperature	Plant part	NO_3-N [mg]	N [mg]
15°C	shoot	0.20	1.17
	root (0-5 cm)	0.12	0.22
	root (5-10 cm)	0.11	0.23
20-10°C	shoot	0.16	1.21
	root (0-5 cm)	0.09	0.25
	root (5-10 cm)	0.11	0.30

App. 11: NO_3-N and total N content [mg] in plant parts at different temperature treatments. Mean is shown, n = 2.

Root temperature	Plant part	Free amino $acids_{total}$ [mg]	Biomass [g fresh weight]
15°C	shoot	0.381	0.254
	root (0-5 cm)	0.516	0.848
	root (5-10 cm)	0.248	0.444
20-10°C	shoot	0.607	0.208
	root (0-5 cm)	5.539	10.88
	root (5-10 cm)	5.154	9.011

App. 12: Total free amino acid content [mg] in plant parts at 15°C RT and 20-10°C RTG and biomass [g fresh weight] of the different plant parts. Mean is shown, n = 2.

Free amino Acid	shoot 15°C	shoot 20-10°C
ASP	104.32	84.35
GLU	164.62	130.85
SER	32.34	64.62
ASN	1.41	11.10
GLN	21.75	87.60
TYR	0.45	2.75
GLY	2.20	9.40
ALA	38.84	89.93
PRO	1.18	43.18
MET	-	-
VAL	2.88	13.50
PHE	2.83	8.74
LEU	1.69	14.24
ILE	1.26	9.25
TRP	0.06	2.19
LYS	2.15	11.65
HIS	1.30	8.25
ARG	1.95	15.87

App. 13: Content of individual free amino acids [µg] in shoots of plants grown at 15°C RT and 20-10°C RTG. Mean is shown, n = 2.

C Further results of plants used in the nutrient uptake kinetics experiment

Root temperature treatment [°C]	Biomass [mg dry weight]		N concentration [% dry weight]		Mg concentration [% dry weight]	
	Shoot	Root	Shoot	Root	Shoot	Root
10	63.42	15.55	4.77[a]	4.16[a]	0.39[a]	0.68
15	61.25	15.23	5.02[b]	4.53[b]	0.40[a]	0.70
20	70.54	15.23	5.16[b]	3.87[a]	0.53[b]	0.62

App. 14: Biomass, N and Mg concentration of plants after 9 h labeling analyzed for nutrient uptake kinetics (hydroponics). Mean is shown. Shoots and roots were separately compared, statistical analysis was done by one way ANOVA and t-test; $p < 0.05$; $n = 3$. Different letters mark statistically significant differences.

D Further results of plants grown in plant pots at room temperature before labeling

	Biomass [mg dry matter]					
Root temperature treatment [°C]	A Shoot	B Shoot	P	A Root	B Root	P
10	111.44	118.50	n.s	65.92[a]	71.25[ab]	n.s.
15	98.36	99.36	n.s.	65.00[ab]	60.34[a]	n.s.
20	97.95	95.71	n.s.	62.08[ab]	89.99[b]	0.025
20-10	101.03	108.07	n.s.	46.31[b]	70.69[ab]	< 0.001
P	n.s.	n.s.		0.031	0.043	

App. 15: Biomass of plants analyzed in labeling experiment in sand. All plants were grown at room temperature before exposing to different root temperatures at labeling. A = label in 1 cm depth; B = label in 6 cm depth. Mean is shown. Statistical analysis was done by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences; n.s. = not significantly different.

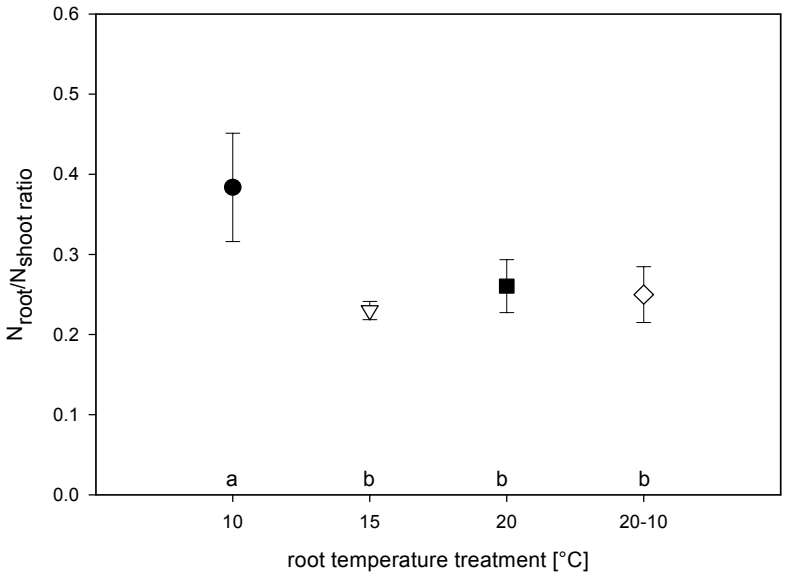
	N concentration [weight-%]			Mg concentration [weight-%]		
Root temperature treatment [°C]	A Shoot	B Shoot	P	A Shoot	B Shoot	P
10	5.20[a]	5.20[a]	n.s.	0.51[ab]	0.45	n.s.
15	4.18[b]	4.23[b]	n.s.	0.55[a]	0.73	n.s.
20	4.64[ab]	4.39[b]	n.s.	0.42[b]	0.37	n.s.
20-10	4.25[b]	4.43[b]	n.s.	0.42[b]	0.36	n.s.
P	0.002	0.042		0.011	n.s.	

App. 16: N and Mg concentration [% dry weight] of plants analyzed 9 h after labeling in sand experiments. All plants were grown at room temperature before exposing to different root temperatures at labeling. A = label in 1 cm depth; B = label in 6 cm depth. Mean is shown. Statistical analysis was done by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences; n.s. = not significantly different.

E Further results of plants grown in plant pots at different root temperature treatments and used for labeling experiments

	Biomass [mg dry matter]					
	A			B		
Root temperature treatment [°C]	Shoot	Root	Root/ Shoot ratio	Shoot	Root	Root/ Shoot ratio
10	207.4[a]	81.6[a]	0.39[a]	187.6[a]	85.1[a]	0.45[a]
15	414.9[b]	143.8[b]	0.34[b]	522.9[b]	213.1[b]	0.41[a]
20	742.0[c]	412.6[c]	0.55[c]	560.5[b]	475.6[c]	0.83[b]
20-10	1738.9[d]	2138.3[d]	1.20[d]	2040.2[c]	2020.1[d]	1.01[b]

App. 17 Biomass of plants analyzed in labeling experiment in sand. All plants were grown at different root temperature treatments. A = label in 1 cm depth; B = label in 6 cm depth. Mean is shown. Statistical analysis was done by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences.



App. 18: N allocation to root and shoot in plants at different root temperature treatments. Mean and standard deviation are shown. Statistical analysis was done by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences.

	N concentration [weight-%]			**Mg concentration [weight-%]**		
Root temperature treatment [°C]	**A Shoot**	**B Shoot**	**P**	**A Shoot**	**B Shoot**	**P**
10	5.06^{a}	4.98^{a}	n.s.	0.20^{a}	0.19^{a}	n.s.
15	5.12^{a}	5.04^{b}	n.s.	0.27^{ac}	0.35^{bc}	0.006
20	4.70^{b}	4.62^{ac}	n.s.	0.43^{b}	0.38^{b}	n.s.
20-10	4.72^{b}	3.90^{d}	< 0.001	0.33^{c}	0.29^{c}	n.s.
P	< 0.05	< 0.05		< 0.05	< 0.001	

App. 19: N and Mg concentration [% of dry weight] of plants analyzed in labeling experiments in sand. All plants were grown at different root temperature treatments. A = label in 1 cm depth; B = label in 6 cm depth. Mean is shown. Statistical analysis was done by one way ANOVA and t-test; $p < 0.05$; $n = 8$. Different letters mark statistically significant differences; n.s. = not significantly different.

F Results of plants grown at 15-3.5°C vertical root temperature gradient

Biomass [mg dry weight]	
Plant part	**15-3.5°C**
Total plant	3166.00
Shoot	1547.87
Root	
total	1618.25
0-5 cm	1597.08
5-10 cm	21.05
10-15 cm	0.04
15-20 cm	n.a.
Root/shoot ratio	0.91

App. 20: Biomass and root/shoot biomass ratio of plants grown at 15-3.5°C vertical root temperature gradient. Mean is shown, n = 7; n.a. = not available.

Abbreviations

∇T	spatial gradient of temperature
ALA	alanine
ANOVA	Analysis of Variance
ARG	arginine
ASN	asparagine
ASP	aspartate
C_{root}/C_{shoot}	Carbon content in roots / Carbon content in shoots
D	dry weight factor
DW	dry weight
DW_{cut}	dry weight of cut off plant parts
f_w, f_s, f_a	volume fraction of water, solids, air
FW	fresh weight
FW_{cut}	fresh weight of cut off plant parts
FW_{-cut}	fresh weight (without cut off plant parts)
GLN	glutamine
GLU	glutamate
GLY	glycine
HEPES	N-(2-Hydroxyethyl)-piperazine-N'-(2-ethansulfone acid)
HIS	histidine
ILE	isoleucine
k_s, k_a	ratio between the space average of the temperature gradient in the solid relative to the water phase, the corresponding ratio for the gradients in the air and water phases
LA	leaf area
LEU	leucine
LYS	lydine
MET	methionine
N_{root}/N_{shoot}	Nitrogen content in roots / Nitrogen content in shoots
PD	density of a blank sheet of paper
PHE	phenylalanine
PRO	proline
PVC	polyvinylchloride

PW	weight of paper leaf
q_h	thermal flux (amount of heat conducted across a unit cross-sectional area in unit time)
r.h.	relative humidity
RGR	Relative Growth Rate
RT	uniform Root Temperature
RTG	vertical Root Temperature Gradient
SER	serine
TRP	tryptophan
TYR	tyrosine
v/v	volume per volume
VAL	valine
w/v	weight per volume
κ	thermal conductivity
κ_c	composite (soil) thermal conductivity
κ_w, κ_s, κ_a	thermal conductivity of water, solids (average value), air

List of Figures

List of Tables

Die hier vorliegende Dissertation habe ich eigenhändig und ohne unerlaubte Hilfe angefertigt. Die Dissertation wurde in vorliegender oder ähnlicher Form noch bei keiner anderen Institution eingereicht. Ich habe bisher keine erfolglosen Promotionsversuche unternommen.

Bonn, 13. August 2007