Chapter 1

Prolog

The world's population presently experiences a period of unprecedented growth, increasing the need to further intensify the agricultural production (Garnett et al. 2013). At the same time, recent climate trends and model predictions suggest shifting temperature, precipitation and circulation patterns on global and regional scales (Shepherd 2014). In this context, the risk of temporal or unseasonal drought is increasing in many regions worldwide (Pachauri et al. 2014). Agriculture accounts for about 75 % of human water use (Wallace 2000). However, the availability of water is the most limiting abiotic factor for plant production (Boyer 1996). Therefore, agricultural production and food security are highly susceptible to increased incidences of drought. Improving the water-use efficiency (WUE) of crop plants and cropping systems is therefore an important strategy to face the current challenges of global change (Pinstrup-Andersen et al. 1999). Higher WUE can be achieved by certain crop management practices like optimized plant nutrition (Blum 2009). As one of the major mineral nutrients potassium (K) is crucially involved in the optimization of plant-water-relations and is reported to considerably enhance the WUE of crops (Grzebisz et al. 2013) as well as their tolerance against environmental stress situations (Cakmak 2005).

1.1   The concept of water-use efficiency

Crop WUE generally relates the amount of CO_2 that is assimilated by a crop plant or a cropping system over a given period of time to the amount of water that is transpired by the system (Viets 1962, Tallec et al. 2013). WUE can be expressed on various spatiotemporal scales (Tambussi et al. 2007). Three general classifications of WUE can be made, which mainly relate to the scale of temporal integration: *instantaneous*, *intrinsic* and *integrated WUE*. The *instantaneous WUE* relates the actual rate of carbon exchange to the rate of transpiration of a plant system.

Instantaneous WUE is very sensitive to short-term fluctuations in environmental conditions. Changes in the atmospheric vapor pressure deficit (VPD) alter the amount of water transpired through the stomata without affecting assimilation. To account for these variations, transpiration can be normalized on the evaporative demand of the atmosphere (Farquhar et al. 1989). The resulting *intrinsic WUE* is robust against short-termed atmospheric fluctuations and is therefore stable over a wide range of environmental conditions (Bacon 2004). However, both instantaneous and intrinsic WUE are subject to diurnal as well as seasonal variations, and might therefore only poorly correlate to the long-term average WUE of a plant system (Medrano et al. 2015). Time-integrated measures of WUE can account for this problem by providing an *integrated WUE* of a plant system over a longer period of development or over the entire duration of growth (Farquhar and Richards 1984).

Moreover, WUE can not only be expressed on different temporal scales, but is also variable regarding the spatial scale of integration. It can be described from the level of individual photosynthetic tissues to ecosystems. In present study, we express WUE on three fundamentally different levels: the WUE of individual leaves (i.e. leaf WUE, WUE_L), of single plants (i.e. plant WUE, WUE_P) and of agricultural crop stands (i.e. ecosystem WUE, WUE_{Eco}; or yield-based WUE, WUE_{Yield}).

1.1.1 Water-use efficiency on the leaf level

The simplest approach to define WUE_L is as the instantaneous ratio of carbon assimilation (A) to transpiration (E) during photosynthetic leaf gas exchange regulated by the stomata. The underlying concept is to consider A and E as processes that can be reduced to the diffusion of CO_2 and water vapor in analogy to Fick's law (Farquhar and Richards 1984):

(Eq. 1.1)

$$WUE_{L, instantaneous} = \frac{A}{E} = \frac{g_c\,(C_a - C_i)}{g_s\,(w_a - w_i)} = \frac{c_a\,(1 - \frac{C_i}{C_a})}{1.6\,\Delta VPD}$$

Here, WUE_L is a function of the stomatal conductance to CO_2 (g_c) and water vapor (g_s), and of the differences between the atmospheric and leaf-internal concentrations of CO_2 (C_a and C_i) and water vapor (w_a and w_i). It is assumed that $g_c = g_s/1.6$ and the gradient in water vapor concentrations is best expressed by the gradient in VPD (ΔVPD). As already introduced, a robust, intrinsic measure of WUE_L requires normalization of E on atmospheric vapor demand ($E/\Delta VPD = g_s$, von Caemmerer and Farquhar 1981):

(Eq. 1.2)

$$WUE_{L, intrinsic} = \frac{A}{g_s} = \frac{C_a\,(1 - \frac{C_i}{C_a})}{1.6}$$

WUE_L is most often calculated from measurements of leaf gas exchange by enclosing a small portion of leaf area in a closed cuvette system. A flow of air is cycled through the cuvette and the difference in CO_2 and water vapor concentration at the inlet and the outlet of the cuvette are measured. The difference in CO_2 concentrations origins not only from A, but also includes the release of CO_2 due to mitochondrial day respiration (R_d). The two processes are difficult to separate, and therefore the apparent net assimilation ($A_N = A - R_d$) is commonly used for calculating WUE_L.

From Eq. 1.2 it is obvious that the major determinant of intrinsic WUE_L is the ratio of C_i to C_a. This fact is very relevant because it allows a time-integrated measure of WUE_L to be derived from the ratio of the stable carbon isotopes ^{13}C to ^{12}C ($\delta^{13}C$) that is fixed in the dry matter of the photosynthetically active organ.

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About 99 % of CO_2 in the atmosphere comprise of ^{12}C and only about 1 % of ^{13}C. The atmospheric $\delta^{13}C$ signature is approximately -8 ‰. During photosynthesis, plants fix CO_2 and incorporate the respective carbon isotopes into their dry matter. $\delta^{13}C$ in the dry matter of C_3 plants ranges around -27 ‰ and is therefore substantially lower than atmospheric $\delta^{13}C$. During the diffusion of CO_2 from the atmosphere into the chloroplasts and during photosynthesis itself a fractionation occurs; the lighter $^{12}CO_2$ is preferred and the heavier $^{13}CO_2$ is discriminated. According to Farquhar et al. (1982), the discrimination of ^{13}C (Δ) is linearly related to the C_i/C_a ratio:

(Eq. 1.3)

$$\Delta = a + (b' - a)\frac{C_i}{C_a}$$

where a and b' denote the discrimination during diffusion through the stomata (4.4 ‰, O'Leary 1981) and by RubisCo (27 ‰, Farquhar and Richards 1984). Δ can therefore directly be linked to the intrinsic WUE_L by combining Eq. 1.2 and Eq. 1.3:

(Eq. 1.4)

$$WUE_{L,\ intrinsic} = \frac{A}{g_s} = \frac{C_a}{1.6}\frac{b' - \Delta}{b' - a}$$

However, two important processes in photosynthetic CO_2 exchange are neglected in this linear model: discrimination against ^{13}C during the diffusion of CO_2 through the leaf mesophyll (expressed as mesophyll conductance, g_m) and during photorespiration. A detailed review on how to integrate these processes into the concept of carbon isotope discrimination and WUE is given by Seibt et al. (2008).

1.1.2 Water-use efficiency on the whole plant level

WUE_P, like WUE_L, is fundamentally linked to the ratio of A to E. However, it additionally requires consideration of sources of carbon and water loss that are not associated with photosynthesis (Farquhar et al. 1989):

(Eq. 1.5)

$$WUE_P = \frac{A\,(1-\Phi_C)}{E\,(1+\Phi_W)}$$

Here, Φ_C is the proportion of carbon fixed during the day that is lost by mitochondrial respiration or due to exudation of organic compounds by the roots. Φ_W represents the proportion of water that is lost 'unproductively', e.g., by transpiration from non-photosynthetic organs or by nocturnal transpiration through partially open stomata. It is hardly possible to assess WUE_P of crops under field conditions, because crops grow in closed canopies and, additionally, the separation of root respiration and heterotrophic respiration from soil organisms is difficult. Integrated WUE_P can, however, easily be measured for single plants grown in pot experiments by relating the plant dry matter at harvest to the total amount of water consumed during the period of growth (Tränkner et al. 2016). The water consumption can be determined by regular weighing of the pots.

1.1.3 Water-use efficiency of agroecosystems

On the level of agricultural crop stands, additional parameters that are relevant for the interaction between plant and soils systems have to be included into the concept of WUE. Instantaneous WUE_{Eco} can thus be expressed as the ratio of net ecosystem CO_2 exchange (NEE) to evapotranspiration (ET):

(Eq. 1.6)

$$WUE_{Eco,\ instantaneous} = \frac{NEE}{ET}$$

Here, NEE is the balance between CO_2 that is assimilated by the specific agroecosystem or crop stand and CO_2 that is released by the sum of plant respiration and heterotrophic respiration of soil organisms. ET is the sum of plant transpiration and direct evaporation from the soil surface. Instantaneous WUE_{Eco} is a relevant parameter from an ecophysiological perspective, but very sensitive to environmental factors like incident light, VPD, temperature and wind.

Assessing the long-term average WUE of an agroecosystem is highly relevant to farmers and agronomists. In an integrated approach, WUE can be based on the relation between biomass accumulation and the total sum of ET of an individual crop stand over the entire growing season. Biomass is easy to measure at harvest and can be expressed either as total dry matter yield or as yield of the economical product (e.g., grain yield for cereals or white sugar yield for sugar beet). Estimating ET is more challenging. Whereas an estimation of potential ET can be easily made from microclimate data using e.g. the Penman-Monteith equation, additional parameters of crop stand development (soil cover, canopy height, leaf area index) and crop-specific coefficients have to be included to calculate the actual ET of a crop canopy on a daily basis (Allen et al. 1998). Summing up the daily values over the growing period will provide the crop-specific in-season ET (Hauer et al. 2015). A simpler estimation of ET that requires less input can be made by balancing soil water storage at sowing or at crop emergence, in-season precipitation and soil water storage at harvest. This calculation provides the cumulative water consumption of a crop stand which can be used as a proxy for ET (Whish et al. 2014). Furthermore, dynamic measurements of ET can be performed by using an Eddy-covariance approach (Zhang et al. 2011). However, due to the large footprint of this method plot-specific measurement cannot be performed in agricultural field trials. Direct

measurements of ET are also possible using lysimeters (Slavík 1974), but these methods are laborious and highly invasive.

Independent of the method applied for estimating the total ET, the integrated yield-based WUE (WUE_{Yield}) is calculated as

(Eq. 1.7)

$$WUE_{Yield} = \frac{Yield}{total\ ET}$$

1.1.4 Perspectives to improve the water-use efficiency in agricultural cropping systems

The overall aim of improving the WUE of crop plants or of agricultural cropping systems requires careful consideration of the different meanings of the term WUE with regard to the various options for spatiotemporal integration.

Several strategies can be followed to enhance the WUE in agricultural systems. For example, innovations in plant biotechnology and plant breeding have developed crop varieties with higher harvest index, achieving higher yields per unit of transpiration (Condon 2004). Improving the plant nutritional status via optimizing fertilization strategies is another major approach to improve crop WUE by optimizing crop productivity and water use (Brouder and Volenec 2008, Waraich et al. 2011). Optimum fertilization with e.g. nitrogen (N), phosphorus (P) and potassium (K) improves the crop's capacity to expand its rooting system and thus absorb soil water more efficiently. It additionally allows a more rapid leaf area expansion, therefore reducing the portion of evaporation from the soil surface and increasing the proportion of water that is used 'productively' by the plant system (Sharma et al. 2015). In the context of WUE, the role of K in the (eco)physiology of crop plants is of specific interest. Adequate K status not only enhances the productivity of crops, K is also a key mineral nutrient critically involved in the balancing of plant-water-relations

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(Marschner 2012) and significantly improves crop tolerance towards environmental stress situations (Cakmak 2005).

1.2 Potassium in plant nutrition

1.2.1 Abundance of K in soils

Potassium (K) is a mineral nutrient required in large quantities by crop plants. When K uptake is not limited, its mass may account for 6 % of the plant dry matter (Leigh and Wyn Jones 1984), but can even exceed 10 % in certain organs of some species, e.g., sunflower (Spear et al. 1978). K represents 2.14 % of the Earth's continental crust, which makes it number eight of the most abundant elements (Wedepohl 1995). In contrast, P and N account for only 757 and 60 ppm, respectively. K is therefore generally the most abundant of the major mineral nutrients in soils (Reitemeier 1951), which generally contain between 0.04 and 3 % K (Sparks 2001). Four distinct forms of K exist in soils: solution, exchangeable, non-exchangeable and mineral K (Barber 1984). 98 % of the total K in the upper 0.2 m of a soil profile is bound in the mineral form. Only 2 % is in the soil solution or in the exchangeable and non-exchangeable phases (Sparks 2001). The balance between exchangeable and non-exchangeable K as well as its slow release from the weathering of minerals play an important role for the plant availability of K in soils and depends on many factors like mineral composition of the parent rock, climate conditions, type and abundance of clay minerals, addition of K fertilizer as well as cropping intensity and -practice (Schröder 1978, Krishnakumari et al. 1984). On a global scale, large areas of arable soils are deficient in K, including two third of the Southern Australian wheat belt (Römheld and Kirkby 2010) and the sandy soils predominating in many parts of Eastern Central Europe, mainly in Poland and Bulgaria (Grzebisz and Diatta 2012). Some of these regions are at increasing risk of temporal drought (Sheffield and Wood 2008, Gassert

et al. 2013), emphasizing the importance of sustainable K fertilization management in order to improve the WUE of the respective agroecosystems and minimize drought-induced yield losses. This, however, requires a fundamental understanding of the functioning of K in the (eco)physiology of crop plants.

1.2.2 Functions of potassium in the plant system and its implications on WUE

Plants take up K only in its ionic form (K^+). K is not integrated into the structure of biomolecules, but rather remains in the plant as K^+ or forms only weak complexes, which makes it very mobile within the plant system (Marschner 2012). This high potential for translocation is essential for many of its physiological functions. K is the major inorganic solute in plants and is essential for establishing gradients in osmotic potential (Ψ_π) within or between individual cells and tissues Therefore, K^+ concentration, together with associated inorganic or organic anions, is a crucial determinant of turgor pressure and involved in important turgor-driven processes like cell growth (Mengel and Arneke 1982) and stomatal movement (Raschke 1975). Furthermore, K^+ highly contributes to maintaining the cytosolic pH in an optimal range for enzyme functioning by activating membrane-bound proton pumping ATPases (Marschner 2012).

During cell growth, K^+ accumulation in cells reduces Ψ_π and initiates a decrease in apoplastic pH. It thus activates enzymes associated with cell wall loosening (Hager 2003). This facilitates the swelling of cells due to an influx of water caused by reduced Ψ_π. Cell growth is essential for rapid leaf area expansion. A reduced rate of leaf area production is one of the first indicators of insufficient K supply (Jordan-Meille and Pellerin 2004), and already mild K deficiency can significantly reduce the leaf area (Gerardeaux et al. 2010).