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The effects of nitrogen supply and water regime on instantaneous WUE, time-integrated WUE and carbon isotope discrimination in winter wheat

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ABSTRACT

Improving water use efficiency (WUE) is important in the water-deficient region in the North China Plain (NCP). How to assess the WUE of different management practices is becoming more important. The aim of the current study was to evaluate the relationship between instantaneous WUE, time-integrated WUE and carbon isotope discrimination ($\Delta^{13}C$) in winter wheat (*Triticum aestivum* L.) using different water and nitrogen (N) supplies. Three water levels and five nitrogen (N) levels with 15 treatments were set up in a pot experiment using PVC tubes with 1 m depth and 20 cm diameter buried in a field for two seasons. Biomass, root weight, grain yield, evapotranspiration (ET), gas exchange parameters and kernel $\Delta^{13}C$ were measured to estimate the plant and leaf WUE. The results showed that the water regime and N supply strongly affected the aboveground and belowground biomass and WUE. The WUE was significantly improved when the N supply was increased from deprivation to higher conditions. When soil evaporation was prevented, the yield of winter wheat was linearly related to ET. The water supply had positive effects, while the N supply had negative effects on the gas exchange parameters. The impact of the interactions between the water regime and N supply on gas exchange parameters was significant, but an interactive effect of the two parameters on final crop yield and WUE was not observed. While both the water regime and the N supply had significant effects on the kernel $\Delta^{13}C$, the water regime had a greater effect on $\Delta^{13}C$ than did the N supply. This study supported the use of the kernel $\Delta^{13}C$ to evaluate crop WUE. However, the relationship between the instantaneous WUE calculated from the gas exchange parameters and the final WUE at yield level was not always consistent. A strong positive relationship between these two factors existed during the grain-fill stage when different management practices implemented their time-integrated effects on the crop growth. No such relationship was found during other growing stages. This study verifies that gas exchange WUE values at the grain-filling stage might provide a tool to assess the final WUE of different management practices.

1. Introduction

The North China Plain (NCP) is one of the most important grain production regions in China. Water shortage has become more serious, restricting agricultural development. Much research has focused on this issue and has indicated that improving the water use efficiency (WUE) during grain production is one of the most important aspects in solving the water deficit problem in the NCP. Measures have been introduced to improve WUE, such as deficient irrigation, mulching and using better cultivars (e.g., Zhang et al., 2003, 2005, 2008, 2010; Fang et al., 2010). Determining the best manner in which to assess the WUE of different management practices is becoming very important.

WUE at the yield level is usually difficult to accurately determine (Gulías et al., 2012). There are alternative approaches for measuring WUE, such as carbon isotope discrimination for a time-integrated estimation and instantaneous gas exchange at the leaf scale (Fischer, 1981; Condon et al., 2004; Medrano et al., 2010; Rizza et al., 2012; Gulías et al., 2012). Instantaneous gas exchange on a photosynthetic basis can be defined by two methods: instantaneous WUE (WUE_{ins}), which is defined as the moles of CO_2 absorbed per mol of H_2O lost through transpiration, and intrinsic WUE (WUE_{int}), which is defined as the net photosynthesis divided by stomatal conductance (Ali and Talukder, 2008; Qiu et al., 2008). Measuring these two parameters is relatively easy and rapid. However, these measurements occur instantaneously and only on a

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portion of the plants; determining whether the WUE at the leaf level could substitute for the WUE at the yield level still needs consideration. Gulías et al. (2012) found that the leaf WUE parameters did not show a consistent relationship with the plant WUE parameter in two perennial grasses. Tomás et al. (2012) studied the WUE of grape cultivars and found that the WUE at the whole plant level of different grapevine cultivars could not be described by leaf-level indicators of WUE.

The carbon isotope composition of plant dry matter ($\delta^{13}C$), which is frequently expressed as the discrimination value ($\Delta^{13}C$), provides a time-integrated measurement of the plant's transpiration efficiency over the period during which dry matter is assimilated; thus, this parameter has been proposed to be an indicator of WUE (Farquhar and Richards, 1984; Farquhar et al., 1989). In C_3 plants, $\Delta^{13}C$ has been used to evaluate drought stress (Condon et al., 1992) because, under conditions in which the plant is not water-stressed, the stomatal conductance is high and CO_2 diffusion in and out of the leaf is relatively free. Under these conditions, Rubisco, the leaf protein that plays a major role in carbon assimilation, preferentially fixes $^{12}CO_2$, and the fixed $^{12}CO_2$ becomes depleted in ^{13}C (Farquhar et al., 1989). Therefore, any factors that can influence the ratio of intercellular CO_2 and atmospheric CO_2 (C_i/C_a), especially N application, have the potential to influence $\Delta^{13}C$ (Clay et al., 2001). Many studies have reported the effects of water stress on WUE and $\Delta^{13}C$; however, the effects of the N supply on $\Delta^{13}C$ and the interaction between the N supply and the water regime remain unclear (Cabrera-Bosquet et al., 2007), so further study was needed.

The North China Plain is facing a serious water shortage problem, while, at the same time, excessive N fertilization is becoming common in this region and may potentially exert more pressure on the environment (Zhao et al., 2006). Therefore, it is important to improve the WUE through N and water regulations. While a moderate water deficiency has been widely reported to have a positive effect on the WUE (Qiu et al., 2008; Zhang et al., 2005, 2008), several studies have shown that the N supply enhances plant productivity by improving the WUE (Lajtha and Whitford, 1989; Shangguan et al., 2000). However, contradictory results have been found regarding the effects of the N supply on the WUE of plants (Mitchell and Hinckley, 1993). Ripullone et al. (2004) reported that differences in N effects on the WUE may be attributed to differences in experimental procedures, such as (a) the way the WUE is estimated (instantaneous measurements versus measurements integrated over long periods); (b) the material studied (individual leaves versus the whole plant); and (c) the experimental conditions (e.g., optimal or limiting water supply). To our knowledge, research on the WUE of winter wheat in the NCP has mostly focused on grain yield level (Qiu et al., 2008); the effects of different water regimes and N supply on WUE simultaneously at the leaf photosynthesis level, biomass level and yield level and their relations were still not clear.

The aims of this study were as follows: (1) to study the combined effects of different water regimes and N supply on the WUE of winter wheat; (2) to verify whether or not gas exchange WUE values could provide an accurate prediction of WUE values obtained from gravimetric analysis; and (3) to examine the combined effects of different water regimes and N supply on $\Delta^{13}C$.

2. Materials and methods

2.1. Experimental site and design

The study was conducted in two winter wheat growing seasons (2010–2011 and 2011–2012) at the Luancheng Agro-Eco-Experimental Station of the Chinese Academy of Sciences, which is located in the northern part of the NCP at the base of Mt. Taihang (37°53,N, 114°40,E; 50 m above sea level). Winter wheat and summer maize are grown there annually as a common crop-rotation system.

The study was conducted using a pot experiment. The pots were PVC tubes 1 m in depth and 20 cm in diameter, with the bottom of each tube being sealed with plastic film and buried in a winter wheat field and the top of the tubes being flat with the surrounding field. A movable canopy was used to prevent rainfall to the tubes. When there was no rainfall, the shelter was moved away from the field. There were 15 treatments with five levels of N application rates and three levels of irrigation. Irrigation was carried out based on the stages of crop development after over-wintering. The nitrogen and irrigation applied to each treatment are listed in Table 1. In the 2011–2012 season, an additional 60 mm of irrigation was added to each tube before winter dormancy. Tubes with different treatments were randomly displayed. Each treatment was replicated six times in 2010–2011 and four times in 2011–2012. There were 90 tubes used in the 2010–2011 season and 60 tubes used in the 2011–2012 season. The soil was taken from a nearby field in which N fertilizer had not been applied for several years. The initial nutrient contents for the soil were 50.1 mg/kg available N, 15.2 mg/kg available P, 114.2 mg/kg available K, 0.67 g/kg total nitrogen and 8.2 g/kg organic matter in the 2010–2011 season. The nutrient contents were 31.2 mg/kg available N, 13.1 mg/kg available P, 109.1 mg/kg available K, 0.51 g/kg total nitrogen and 6.2 g/kg organic matter in the 2011–2012 season. The initial soil nutrient contents in the 2010–2011 season were higher than those of the 2011–2012 season.

Before filling the tubes, the soil was mixed with different amounts of N as listed in Table 1. In each tube, $Ca(H_2PO_4)_2$ (0.016 kg per tube, P_2O_5 content is 12%) and KCl (0.006 kg per tube, K_2O content is 50%) were applied as base fertilizers mixed with the soil before packing. The soil bulk density was maintained at 1.3 g/cm^3. The initial soil moisture was maintained at 27.3% volumetric water content, which was 85% of the field capacity. The tubes were planted with 20 seeds of winter wheat (Cultivar KN199). After seed emergency, the surface of the soil was covered with 3–4 cm of fine sand to prevent soil evaporation. According to Yuan et al. (2009), the fine sand could prevent soil evaporation by more than 80%. Before each irrigation, the sand was removed and after irrigation, the sand was moved back immediately. So the sand was always kept dry, which also minimize soil evaporation loss. When the seedlings had approximately 3 leaves, thinning was conducted and each tube was limited to 17 plants, which was the density of the surrounding field.

2.2. Measurements

2.2.1. Weather conditions

Weather data from an automatic weather station approximately 100 m away from the experimental site were used. The recording of the main weather factors included temperature, humidity, wind speed, radiation and rainfall at hourly intervals. The daily average temperature (T), net radiation and vapor pressure deficit (VPD) were shown in Fig. 1. The two seasons had similar climatic conditions. However, the lower temperature during the recovery stage and the fast increase in temperature during jointing and earlier grainfill stages in 2011–2012 season negatively affected crop production.

2.2.2. Aboveground dry matter, grain yield and total root weight

The aboveground dry matter was measured at harvest. All of the plants were cut at the base and air-dried to a constant weight prior to recording the weight. The grain was then separated, and its weight was recorded. The PVC tubes were split carefully with a