Development of nitrogen dilution curves for current Australian wheat varieties

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Abstract
Accurate nitrogen (N) application rates are of major importance in agriculture. While fertiliser guidelines have improved, the relationship between yield and N rates remain the main reference. However, this relationship is highly variable with soil, variety and season. A better reference is a 'nitrogen dilution curve' which relates the critical N concentration of wheat shoots (i.e. the lowest concentration of N needed to achieve maximum growth and (theoretically) maximum yield), with biomass. Crops can be benchmarked against this curve to determine their N status, i.e. assess if N fertiliser is applied at the correct rate, or if the crop is over- or under-fertilised. Nitrogen dilution curves exist for winter wheat, but these are developed for irrigated (non-drought stressed) crops and for older (European and Asian) wheat varieties. In South Australia, winter wheat is grown under rain-fed conditions where water and N may co-limit biomass production. In addition, breeding for grain yield has shifted the biomass-nitrogen balance over the past 20 years and the original dilution curve needs to be updated. Here we introduce the GRDC funded project "Benchmarking wheat yield against nitrogen use" (2014-2017). During this project N dilution curves will be produced for current wheat varieties under irrigated and rain-fed conditions. We will assess how drought stress may change the parameters of the curves and how this affects yield. The curves can be used to benchmark wheat in both growers' fields and National Variety Trials. Here we present preliminary results from the first year (2014) of the project and outline the trials for the next two years.

Key words
N status, Nitrogen Nutrition Index, SPAD, Greenseeker, ^{13}C analysis, diagnostic tools.

Introduction
Accurate nitrogen (N) application rates are of major importance in agriculture. Over-application of N can lead to less productive crops and environmental pollution. Continued under-fertilisation leads to soil N mining and yield gaps. In South Australia, farmers are often conservative in their use of N fertiliser because of uncertain rainfall and associated (financial) risks. Consequently, is not uncommon that in seasons with average or above average rainfall, wheat crops in South Australia experience some degree of N deficiency (Sadras 2002).

Despite the importance of N for crop yield and its incidence in the variable costs of farm businesses, there is no established benchmark to assess the N status of crops. Fertiliser guidelines have improved over recent years, with emerging technologies based on canopy size and greenness. However, yield-N relationships remain the main reference. A major limitation of this approach is that yield-N relations are highly variable with soil, crop and season (Sadras and Lemaire 2014).

There is a strong relationship between crop biomass and crop N content. As the crop matures there is a reduction in the levels of N concentration requirements to achieve maximum plant growth (Greenwood et al. 1990). This happens for several reasons. Young crops are mostly leaves, with high N concentration for photosynthetic processes. After stem elongation, the leaf:stem proportion declines, and the lower N concentration in stems drives the whole-shoot N concentration down (Lemaire *et al*. 2008). In addition, as the crop grows, N is relocated from shaded leaves in lower position of the canopy to well-illuminated leaves at the top of the canopy (Hikosaka 2005). The concentration of N in a plant is thus related to its actual biomass. If, due to for example, sowing date or wheat variety, wheat biomass in one trial is lower than in another trial, N concentrations cannot be effectively compared because each crop requires a different amount of N to achieve maximum growth. In order to assess the N status of a crop, we must account for crop biomass.

Nitrogen Dilution Curve and Nitrogen Nutrition Index
To account for crop biomass when determining the N status of a crop, a 'nitrogen dilution curve' and 'nitrogen nutrition index' (NNI) should be used (Lemaire and Meynard 1997). A nitrogen dilution curve shows the minimal concentration of N in shoots that is required to achieve maximum growth, i.e. the *critical N concentration*, plotted against biomass. Wheat crops can be benchmarked against this curve by deriving the NNI. The NNI is calculated by dividing the actual N concentration of the crop at a given biomass, by the critical N concentration derived from the N dilution curve at that same biomass. If the NNI is 1, then the actual N concentration is at the critical level and growth is maximum. When the NNI is < 1, this indicates that the crop may be N deficient while higher than 1 means the increase in N uptake does not increase crop growth and is "luxury" consumption of N.

Nitrogen dilution curves have been developed for irrigated winter wheat by e.g. Justes *et al*. (1994) in France and Zhao *et al*. (2014) in China. In South Australia however, winter wheat is grown under rain-fed conditions where water and N may co-limit biomass production (Sadras 2005). Further, new wheat varieties in Australia have an increased capacity to uptake N from the soil (Sadras and Lawson 2012). New wheat varieties may therefore have different critical N and higher fertiliser requirements. In this GRDC funded project, we will produce a new nitrogen dilution curve for current Australian wheat varieties in rain-fed systems under Mediterranean climate conditions. We will also assess how drought stress affects the parameters for the N dilution curve. The new nitrogen dilution curve can help improve diagnostic criteria and management of N fertiliser application in both growers' fields and National Variety Trials (NVT).

Here we present a subset of the results from the first year of the project. We also outline the rest of the project in the "Trials 2015-2016" section.

Methods
In 2014, a full factorial trial was established with four current wheat varieties (Mace, RAC1843, Scout and Trojan) and five N fertiliser rates (0, 60, and split applications totalling 120, 180, 240 kg-N/ha) at two locations in South Australia, Hart (33°45'10 S, 138°23'51 E, red dermosol) and Turretfield (34°32'32 S, 138°47'20 E, red brown earth). Total rainfall in the April-October season was about average for both Hart and Turretfield (±350 and 270 mm respectively), however both sites had relatively dry endings of the season. Turretfield was sown about 2 weeks later than Hart.

Aboveground biomass was sampled fortnightly starting at tillering to determine biomass and N content. At maturity, biomass was harvested for yield and yield components. A preliminary dilution curve for the 2014 data was fitted following the general procedure of Justes *et al*. (1994) with some modifications: in short, for each location and sampling date, N treatments were compared to determine the point where an increase in N concentration of the biomass did not significantly increase biomass dry weight. The N concentration after which no significant increase in biomass dry weight occurred is the critical N concentration. Statistical analysis was done using one-way ANOVA and Duncans post hoc testing. Not all sampling dates could be used to calculated critical N concentrations due to a lack of significant difference in biomass among the treatments. From the 2014 data, five sampling dates (i,e. 4 data points from Hart and 1 from Turretfield) could be used to construct the preliminary curve (Fig. 1). As this is a limited number, the preliminary dilution curve and related Nitrogen Nutrition Indexes should be interpreted with this in mind.

Results and discussion
A preliminary nitrogen dilution curve was fitted in Figure 1.

Using data from the trials to explore yield responses to NNI, we expected an initial increase in yield with an increase in NNI, yields peaking when the NNI is around 1, and decreasing yields when NNI is larger than 1. At Hart, yield more or less followed the expected trend in relation to NNI (Fig. 2b). Though more obviously for NNI values >1 than <1. At Turretfield yield was highest for NNI's <1, and decreased with an increase in NNI (Fig. 2a). As Turretfield was sown and harvested later than Hart, crops at this location may have been more affected by drought stress at the end of the season, causing the crops with a lower N status to produce higher yields. Because the preliminary curve was constructed mostly with data points from Hart, the

preliminary curve may be better representing crops that are less affected by drought stress. This hypothesis will be tested in the next two seasons of the project (see "Trials 2015-2016" section below).

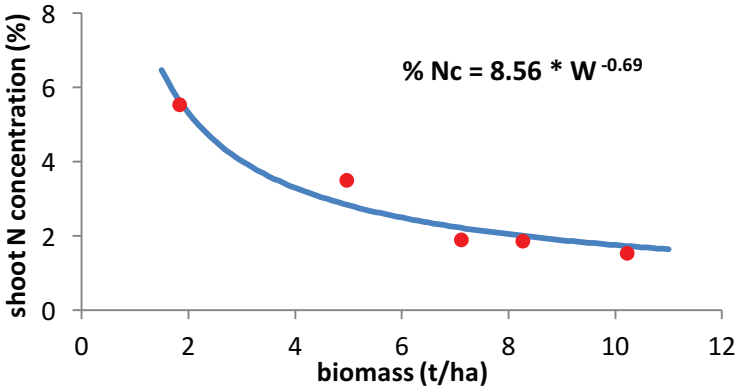

Figure 1. Preliminary nitrogen dilution curve. Red dots are critical N concentrations calculated from the 2014 data and used to fit the dilution curve. The concentration of N in shoots required to achieve maximum growth declines with increasing biomass. Nc: critical nitrogen concentration (%), W: biomass (t/ha).

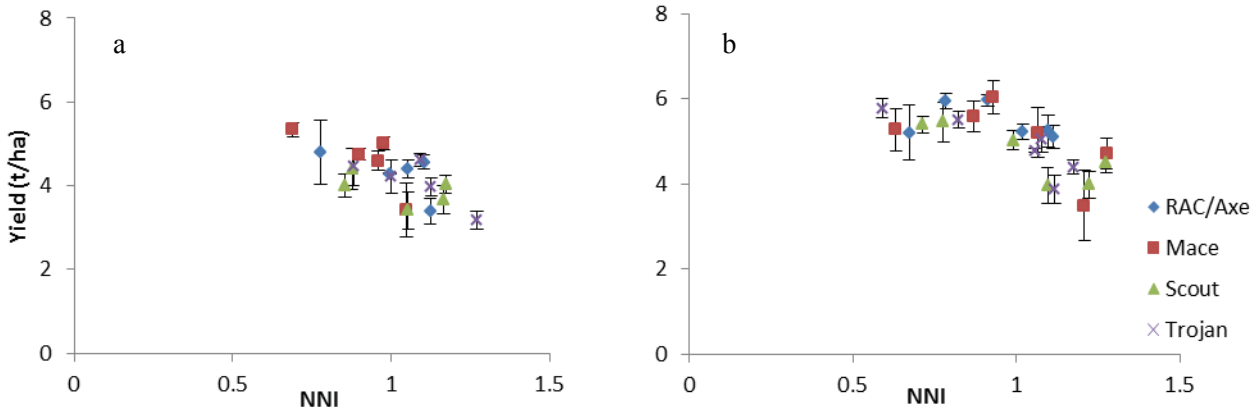

Figure 2. Yield vs. nitrogen nutrition index (NNI) per wheat variety at flowering. (a) Turretfield (blue diamond refers to RAC1843), and (b) Hart (blue diamond refers to Axe). Data points represent the average NNI in each N rate treatment (N = 3).

A reduction in yield under high N rates (NNI > 1) is often reported when high vegetative growth constrains grain fill because soil moisture gets depleted sooner. Interestingly, we did not find a significant increase in total biomass with an increase in N rate (data not shown) but we did find an increase in the leaf:stem ratio. A relatively higher amount of leaf biomass in the high N treatments may still indicate higher transpiration rates and a quicker depletion of soil moisture. Furthermore we found that the reduction in yield was mostly related to 1000-grain weight (as opposed to e.g. grain number), which has previously been found to be caused by high levels of N (Ferrante et al. 2010).

Trials 2015-2016

In the 2015 and 2016 growing seasons, similar full factorial trials will be set up under both rain fed and irrigated conditions. We will use ^{13}C isotope analysis and canopy temperature measurements to assess the degree of drought stress in the rain fed trials compared with the irrigated trials and compare how the N dilution curve parameters change for drought stressed crops.

In order to make the NNI a more practical tool for farmers, we also test two methods to measure proxies for shoot N in the field. We use a SPAD meter that measures chlorophyll content and a handheld Greenseeker to measure crop greenness (normalised difference vegetation index, NDVI).

During all seasons, samples are also collected from commercial farmers' fields and National Variety Trials (NVT) to get an indication of the current N status in crops and NVT in the mid north region in South Australia.

Conclusions

While a preliminary dilution curve was constructed using 2014 data, additional data is needed from the 2015 and 2016 seasons to produce a more robust dilution curve. Using data from the trials planned in 2015 and 2016 for rain fed and irrigated crops, we will assess how drought stress affects the parameters of the dilution curve and use this to make predictions for crop N requirements in years with below average rainfall.

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