Forage crops nitrogen use efficiency changes under drought conditions

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Abstract. Ensuring sustainable agriculture and food security is one of the biggest challenges under changing climate. The frequency and severity of droughts is increasing as a result of climate change. These extreme events may lead to decreased forage crop productivity and financial incomes. Forage crop productivity strongly depends on plants nitrogen use efficiency which in turn may be affected by changed climate conditions. The aim of the study was to examine the influence of drought on the growth and nitrogen use efficiency of alfalfa (*Medicago sativa* L.) and *festulolium* (*Festulolium loliaceum* (Huds.) P.Fourn.) at different rates of nitrogen (N) supply (background, 60 and 90 kg N ha^{-1}). Alfalfa and *festulolium*, grown in a climate-controlled glasshouse, were subjected to short-term drought (7 days) under ambient CO_2 (400 ppm) concentration. After the cease of stress, plants were rehydrated and left for 7 days to recover under control conditions. The growth (aboveground and belowground biomass, height), plant N content, nitrogen use efficiency (uptake (NupE), use (NUE) and utilization (NutE)) of both plant species were evaluated. Drought had an adverse effect on N accumulation and N uptake, use and utilization efficiency in alfalfa and *festulolium*. Additional N supply reduced efficiency of N uptake and use.

Keywords: drought, forage crops, nitrogen supply, nitrogen use efficiency

Introduction

Grasslands are of major importance for the agriculture in Europe, covering about 40% of total agricultural land of Europe and 7% of total area of Northern Europe and the Baltic States region (Dengler *et al.* 2020). Ensuring sustainable production of high-yielding and high-quality forages is critically important for livestock production.

Alfalfa (*Medicago sativa* L.) is the most important forage legume worldwide due to its wide adaptation to different climates and soils, high yield, palatability, and high quality (Putnam & Orloff 2014). *Festulolium* hybrids since their development in the 1970s have become ones of the dominant forage grass species in Europe. Festulolium hybrids have the potential of combining the superior forage quality of ryegrass species (*Lolium*) with the high persistency and stress tolerance of fescues (*Festuca*) (Thomas, Morgan & Humphreys 2003).

Future global climate change may lead to higher temperatures, a longer growing season and more water shortage stress in many locations (Hanssen-Bauer et al. 2009), and this may affect the persistency of grasslands. Drought is listed among the most important abiotic factors limiting plant growth and productivity. The frequency and intensity of droughts is increasing as a result of ongoing climate change (IPCC, 2013). Agricultural drought, defined as deficiency in soil moisture, is of great importance for forage plants production. Soil nitrogen content is a key limiting factor in most agricultural cropping systems and its uptake could be highly affected by the droughts (da Silva, Nogueira, da Silva & de Albuquerque 2011). Improving nitrogen use efficiency of forage plants is of key importance because nitrogen fertilizers represent the major cost in plant production and contributes to soil and water pollution (Masclaux-Daubresse *et al.* 2010). Therefore, the main aim of this study was to evaluate the influence of drought on the growth and nitrogen use efficiency of alfalfa (*Medicago sativa* L.) and *festulolium* (×Festulolium loliaceum (Huds.) P.Fourn.) at different rates of nitrogen supply.

1. Materials and methods

2.1. Plants' growing conditions and experimental design

Alfalfa (*Medicago sativa* L.) and *festulolium* (×*Festulolium loliaceum* (Huds.) P.Fourn.) were sown in pots filled with a mixture of field top-soil, perlite and sand (5:3:2, by volume). Before sowing, the commercial complex fertilizers (NPK 8-19-29) (Achema, Lithuania) were applied. Plants were grown in a climate-controlled glasshouse at 23/14±1 °C (day/night), the relative air humidity (RH) was 55-60%. The photosynthetic active radiation (PAR) was provided by a combination of sunlight and additional lamps (6×400 W) with a photoperiod of 14 h. Plants were watered every second day and volumetric soil water content (SWC) was kept at 30% (Delta-T Devices Ltd., Cambridge, UK).

Three different treatments were conducted to imitate different nitrogen fertilization rates: background level (N0, only background level), moderate (N60, 60 kg ha^{-1}) and high (N90, 90 kg ha^{-1}) fertilization. Additional nitrogen doses were applied as ammonium nitrate.

When plants were at 13-14 growth stage according to the BBCH growth scale, plants were assigned to two different watering regimes: control and drought. Control group soil water content (SWC) was held at 30%. Drought was applied by withholding watering for 7 days and after this period plants were well watered to 30% and left for 7 days for recovery. All treatments were done in triplicate.

Plants were harvested after the recovery period. Aboveground and root biomass were dried at 60 °C until a constant dry weight and weighed. Nitrogen (N) concentrations were determined by combusting in an elemental analyzer Flash 2000 (Thermo Fisher Scientific, Great Britain). Aboveground plant N content was determined as the product of tissue N concentration and biomass.

2.2. Nitrogen use efficiency evaluation

Nitrogen uptake efficiency (NUpE) was calculated as the ratio of total nitrogen in the aboveground tissues to applied nitrogen (Moll, Kamprath & Jackson 1982). Nitrogen use efficiency (NUE) was determined as the total aboveground biomass produced per unit of applied nitrogen (Xu, Fan & Miller 2012). Nitrogen utilization efficiency (NUtE) was calculated as total aboveground biomass divided by the total accumulated N (Abbadi & Gerendás 2015).

2.3. Statistical analysis

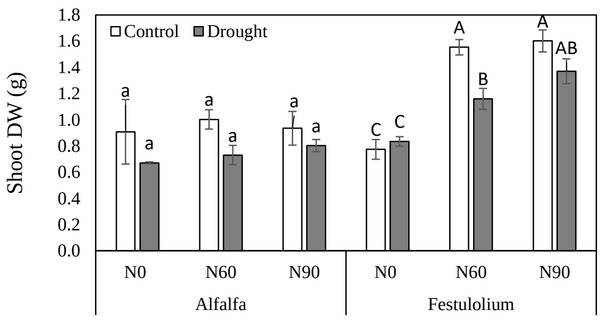
LSD test was applied to evaluate statistically significant differences between the means of investigated parameters between the control and different treatments. Differences were considered to be statistically significant at $p<0.05$. Factorial ANOVA was used to assess the main effects of water regime and nitrogen fertilization treatments and their interaction for the plant response parameters. All analyses were performed by STATISTICA 8 software.

2. Results and discussion

Alfalfa shoot DW was not significantly affected neither by drought stress nor different N supply (ANOVA, $p>0.05$) (Fig. 1). In contrast, the aboveground DW of non-legume *festulolium* was largely affected by water regime and N supply (ANOVA, $p<0.05$), though the interactive effect was insignificant ($p=0.06$). however, reduced alfalfa biomass production was observed in other studies. Suppressed irrigation by 30% has decreased alfalfa biomass production and the stem biomass was mostly affected compared to that of leaves and roots (Aranjuelo, Irigoyen & Sánchez-Díaz 2007). Precipitation amount had the greatest impact on alfalfa yield in different regions of United States (Izaurralde *et al.* 2011). The DW of *festulolium* was substantially enhanced by N addition at normal water regime, leading to about two-fold higher shoot DW under N level of 60 and 90 kg ha^{-1}, compared to N0 ones ($p < 0.05$). Whereas drought stress has reduced this stimulatory N effect and DW in N60 and N90 treatments was by 38.9% and 64.1% higher, respectively. *Festulolium* has shown good regrowth during the recovery after the drought periods in the field study (Cernoch & Kopecky 2020).

Drought has slightly reduced (8%, $p<0.05$) alfalfa shoot height under background N level, though this effect was not observed under additional N supply (Fig. 1). Additional N supply gad no impact on the shoot height of alfalfa and has led to an increased height of *festulolium* shoots ($p<0.05$), though no differences were found among N60 and N90 treatments.

Drought has significantly diminished N content in the alfalfa shoots, though additional N supply had no impact (ANOVA, drought: $p=0.001$, N: $p=0.59$, Drought×N: $p=0.23$) (Fig. 2A). On the contrary, N content in *festulolium* did not responded to the drought and was significantly affected by the additional N supply (ANOVA, drought: $p=0.35$, N: $p<0.01$, Drought×N: $p=0.33$). N content in the *festulolium* increased with additional N (control $r_s=0.69$, drought $r_s=0.95$, $p<0.05$) and was up to 50.0% and 85.8% under normal and restricted irrigation, respectively.



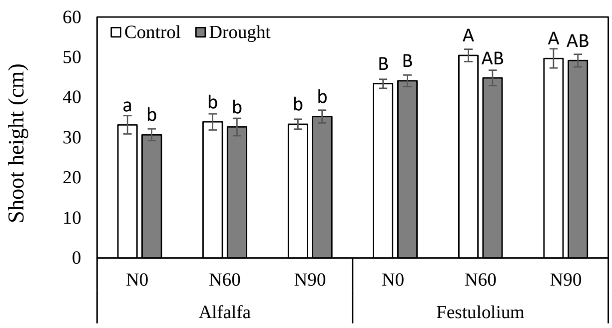


Figure 1. Shoot dry weight and height of well-watered (control) and drought stressed alfalfa (*Medicago sativa* L.) and *festulolium* (×*Festulolium loliaceum* (Huds.) P.Fourn.) grown under different nitrogen supply (N0, N60 and N90)

Alfalfa more efficiently uptakes N from the soil than *festulolium* and this might explain the higher N content in the shoots as well (Fig. 2A, B). However, in both species the highest NUpE was detected at the lowest N soil content. In alfalfa NUpE decreased significantly with N dose both under control and drought conditions (control r_s=-0.90, drought r_s=-0.87, $p<0.05$). NUpE decrease with N increasing N supply was shown in several studies and is controlled by N assimilation, transportation from roots to shoots and plant demand (Nacry, Bouguyon & Gojon 2013; Stahl, Friedt, Wittkop & Snowdon 2016; Perchlik & Tegeder 2017). The decrease in uptake efficiency of other major nutrient phosphorous (P) was detected in safflower (*Carthanus tinctorius* L.) and sunflower (*Helianthus annuus* L.) (Abbadi & Gerendás 2015). Drought has diminished NUpE by alfalfa and the drought effects was more pronounced under higher N supply.

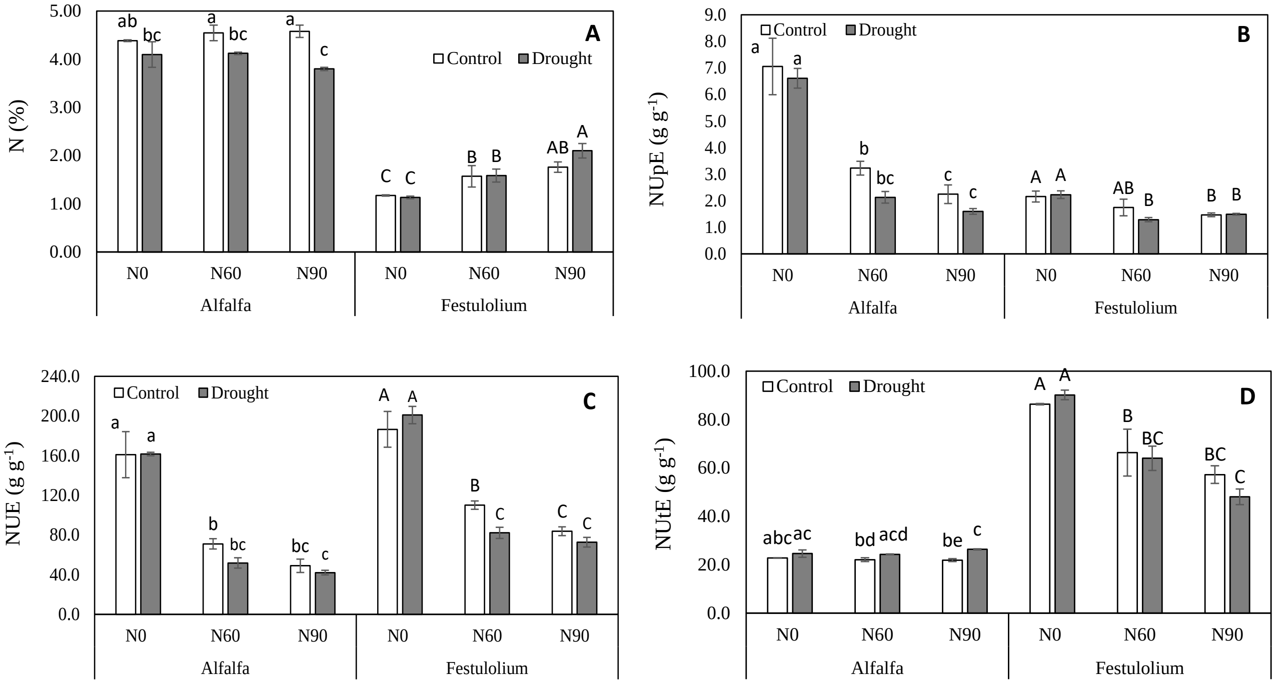


Figure 2. Shoot nitrogen concentration (%N), nitrogen uptake efficiency (NUpE), nitrogen use efficiency (NUE) and nitrogen utilization efficiency (NUtE) by well-watered (control) and drought stressed alfalfa (*Medicago sativa* L.) and *festulolium* (×*Festulolium loliaceum* (Huds.) P.Fourn.) grown under different nitrogen supply (N0, N60 and N90)

Festulolium has produced more aboveground biomass relative to the amount of N supplied, i.e., exhibited higher NUE than alfalfa (Fig. 2C). Drought had no significant effect on the NUE, while N significantly affected the NUE of both species (ANOVA both species, drought: p=0.19, N: $p<0.01$, Drought×N: $p_{alfalfa}$=0.44, $p_{festulolium}$=0.055). NUE was higher in *festulolium* than in alfalfa, though in both species it has significantly declined with N supply dose under normal and drought conditions. However under drought conditions NUE decline with additional N supply was more pronounced than under normal irrigation.

Festuloliom has also utilized more efficiently the accumulated nitrogen, i.e., NUtE was higher in *festulolium* than in alfalfa (Fig. 2D). Alfalfa NUtE was independent of N supply dose (ANOVA, p=0.48), while in *festulolium* it decreased with N dose ($p<0.01$). Interestingly, that alfalfa has utilized N more efficiently (by 7.9-20.3%) under drought conditions (ANOVA, $p<0.001$) than under normal soil moisture content and the efficiency increased with N supply. Reduced NUE and NUtE under higher N supply was also shown in peas (Perchlik & Tegeder 2017), *Rhipsali paradoxa* (Silva, Ferreira, Jocys, Kanashiro & Tavares 2017) and different rice varieties (Wu *et al.* 2016).

The study shows that additional nitrogen supply do not offset the adverse drought impact on forage crops however the response is species-specific. More detailed research is needed to clarify the drought impact on the forage crop nitrogen use efficiency under interspecific competition.

3. Conclusions

This study has demonstrated that drought has reduced the aboveground growth of both species, while additional N supply has led to an increased growth.

Drought had an adverse effect on the N accumulation and N uptake, use and utilization efficiency. Additional N supply has led to a decreased efficiency of N uptake and use in alfalfa and had no impact on its utilization efficiency and shoot content. *Festulolium* has shown a decrease in nitrogen uptake, use and utilization efficiency with increasing N supply, though shoot N content increased with N dose.

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