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RESEARCH ARTICLE

Agronomic performance, nitrogen acquisition and water-use efficiency of the perennial grain crop *Thinopyrum intermedium* in a monoculture and intercropped with alfalfa in Scandinavia

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Abstract
The perennial forage grass *Thinopyrum intermedium* (Host) Barkworth & Dewey, commonly known as intermediate wheatgrass (IWG) or by the commercial name Kernza™, is being developed as a perennial grain crop, i.e. being bred for its improved agronomic performance and food qualities. Intercropping legumes and grasses is a strategy for improving resource use and sustainability in cropping systems. Here, we show for the first time the agronomic performance of IWG as a perennial cereal grown as a monocrop and as an intercrop (alternate row, 0.5:0.5) with *Medicago sativa* L. (alfalfa/lucerne) in southern Sweden. The seeds of cycle 3 IWG were accessed from The Land Institute (TLI) of Salinas, Kansas, USA, and used to establish a local seed production plot (in 2014) for the establishment of the perennial systems (in 2016) utilised in this study. Both the monocrop and intercrop were sown with 25 cm row spacing with alternate rows of IWG and alfalfa in the intercrop (i.e. replacement design) with unknown sowing density. Intercropping provided sustained IWG grain production under the dry conditions of 2018, but also in the following year. This was evidently associated with a higher nitrogen accumulation in intercropped practice. Thus, intercropping seems to have stabilised the IWG grain production in the dry conditions of 2018, when the grain production in the intercrop was similar to that of the monocrop in the same year. This result was further supported by the lower discrimination against ^{13}C (as an indicator of water use efficiency) in the intercrop components compared to the sole crop in 2018. The lower discrimination indicates high water use efficiency in the intercropped IWG in comparison to the IWG in monoculture, and we conclude that intercropping perennial cereal grain crops with legumes provides better growing conditions in terms of nitrogen acquisition, and water status, to cope with more extreme drought spells expected from climate change.

Keywords Intermediate wheatgrass · Grain yield · Straw yield · Drought · N content · N_2 fixation · Lucerne

1 Introduction

Perennial crops represent a paradigm shift in agriculture and have the potential to contribute to increased sustainability of production systems (Crews et al. 2018; FAO 2013). Perennial cereal grain crops are more robust and multifunctional than annual crops (Ryan et al. 2018). In the pursuit for suitable candidates for the development of perennial grain cereal crops, the perennial forage grass *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey, commonly known as intermediate wheatgrass (henceforward referred to as IWG), was selected for domestication in 1983 (Wagoner and Schauer 1990) and included in a breeding programme for perennial cereal grain production in 2002 (DeHaan et al. 2013) and trademarked under the name Kernza™ (Fig. 1). The selection was based on flavour, ease of threshing, large seed size, resistance to shattering, lodging resistance, ease of harvest and perennial growth, and was identified as the most promising species among 100 other perennial grasses (Wagoner and Schauer 1990). While plant breeding improves grain yields, it has been suggested also to focus on the crop multifunctionality, which perennial cereal may provide besides grain production (Duchene et al. 2019). Potential multiple functions from perennial cereals include feed and forage production, protection and regeneration of soil quality, reduced nutrient losses, reduced requirements for agrochemicals, climate change adaptation and mitigation, conservation of biodiversity and

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Fig. 1 Intermediate wheatgrass Kernza™ grains after harvest and threshing in Sweden, 2017. Photograph courtesy of Ryan Davidson.

improved agroecosystem resilience (Ryan et al. 2018). Taking the landscape perspective into account, multifunctionality is vital and the inclusion of elements of perennial crops in the production landscape may provide a higher delivery of several ecosystem services beyond the provisioning service of crop yields (Asbjornsen et al. 2014; Landis 2017) (Fig. 2).

The inclusion of legumes in the cropping system provides a wider range of ecosystem services like the increase in the N use efficiency (NUE) (Jensen et al. 2020), leading to a more sustainable agricultural production. Moreover, biological N_2 fixation reduces the need for synthetic N fertilisers (Jensen et al. 2020) and reduces environmental costs related to fertiliser production, transportation and use, not the least in terms of climate change (Jensen et al. 2012). Intercropping IWG with perennial legumes makes atmospheric N_2 available to the production system via symbiotic N_2 fixation. With time, fixed N in legume residues and exudates are mineralised and made available to the perennial cereal (Crews et al. 2016), while N also may be transferred from legumes via mycorrhizal networks (Johansen and Jensen 1996; Thilakarathna et al. 2016). Niche complementarity is a well-known mechanism driving coexistence resulting in potential over-yielding (Gross et al. 2007). This mechanism often erases or supplements the mutual competition pressure between the legume and cereal crops. It is well-known that intercropping cereals and grain legumes result in higher and more stable grain yields, and a higher cereal protein concentration compared to the sole crop cereals (Bedoussac et al. 2015). However, for the novel production systems including IWG, studies on intercropping in Kernza production are sparse and results contradictory. While Dick et al. (2018) did not find and effect of intercropping with alfalfa (*Medicago sativa*), sweet clover (*Melilotus officinalis*) and white clover (*Trifolium repens*) on Kernza grain production, Tautges et al. (2018) found that the yield loss with stand age was reduced when IWG was intercropped with alfalfa (*Medicago sativa*). Furthermore, IWG has been found to provide

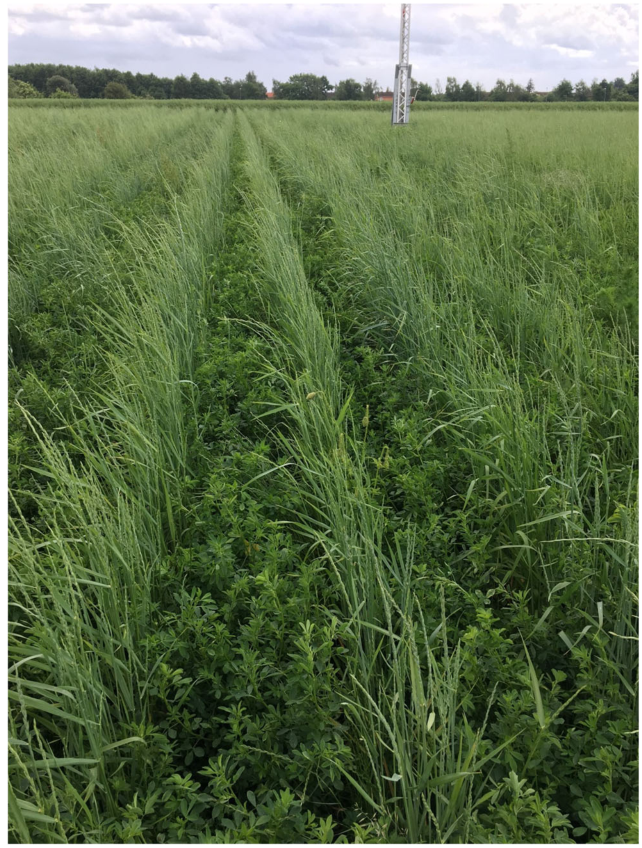

Fig. 2 Intermediate wheatgrass (*Thinopyrum intermedium*) and alfalfa (*Medicago sativa* L. cv. Power 4.2) in 2016 at the SITES Lönnstorp Research Station, Swedish University of Agricultural Sciences (SLU), Alnarp, Sweden. Photograph by Erik Steen Jensen.

suitable forage for beef and dairy cows, as well as for growing heifers, and that intercropping IWG with red clover (*Trifolium pratense*) increased the forage nutritive value in the fall (Favre et al. 2019).

Intermediate wheatgrass has been shown to tolerate partial-season irrigation deficits better than other perennial grasses (Orloff et al. 2016) and to maintain a relatively high water use efficiency during the growing season (Culman et al. 2013; Oliveira et al. 2019). This is an important feature in the current climatic conditions, where drought events are expected to increase in frequency and severity in southern Europe (Roudier et al. 2016), but also for northern Europe (SMHI 2019). The performance of perennial grasses can be related to the capability of sustained aboveground biomass production under dry conditions through enhanced water use efficiency (WUE), which has been proven to occur in C_3 grasses (Kørup et al. 2018), often resulting from the response mechanism of reducing discrimination against ^{13}C in photosynthesis (Mårtensson et al. 2017). In addition, the larger root system of IWG allows access to water in deeper soil layers, while the annual crops do not have access to these resources (Vico and Brunsell 2018). Dry growing conditions make the soil nutrients, especially N, immobile, resulting in reduced

NUE. Indeed, the relationship between crop water use, WUE and grain NUE has been confirmed (Dalal et al. 2013).

To our knowledge, research on agronomy and nitrogen nutrition in IWG and Kernza grain production has not yet been carried out in Scandinavia to any large extent, neither in sole cropping nor in intercropping with perennial legumes. This study is the first one to determine the agronomic performance and nitrogen acquisition of IWG when grown under the temperate climate of southern Sweden with and without a perennial legume companion/service crop during the initial three years. The study also demonstrate the capability of sustained aboveground biomass production under dry conditions through enhanced water use efficiency as indicated by the downregulation of ^{13}C discrimination. To elucidate the effect of intercropping on IWG, we pose the following hypothesis:

> Intercropping IWG with alfalfa will increase IWG grain and straw biomass yields, N concentrations and N accumulated in the IWG biomass, as well as reduce the discrimination against ^{13}C in IWG under dry conditions.

2 Methods and materials

2.1 Experimental design

The SITES (Swedish Infrastructure for Ecosystem Science) Agroecological Field Experiment (SAFE), is a long-term south Swedish cropping system large-scale experimental facility, was established in 2016 on previously conventionally managed land. The SAFE is located at the SITES Lönnstorp Research Station, SLU, in Alnarp (55.65° N, 13.06° E) in a region with a humid continental climate (Fig. 3). The soil type is a sandy loam soil (67% sand, 18% clay), with a soil pH_{H2O} (0–30 cm) of 7.3, and 0.9% soil organic matter content. Soil nutrient conditions for the site correspond to 51 mg kg^{-1} of aluminium lactate extractable P, 0.36 g kg^{-1} total P, 65 mg kg^{-1} aluminium lactate extractable K, 1.4 g kg^{-1} total K and a total N content of 0.2 %. The SAFE has a block design with spatially contained blocks where the geographical layout was guided by initial measurements on soil variables (pH, moisture, nitrogen levels, etc.) to ensure similar conditions within the blocks. The SAFE includes a perennial cereal grain system, representing a model for future potential perennial cereal grain production under low input organic management. The perennial cereal grain system in SAFE holds a monocrop with IWG and an intercrop with IWG and alfalfa (*Medicago sativa* cv. Power 4.2; Fig. 2) in large plots (48 × 50 m). Seeds of intermediate wheatgrass were accessed from the cycle 3 (2014) germplasm of the perennial grain breeding program of The Land Institute (TLI) of Salinas, Kansas, USA (Zhang et al. 2016). The seeds were used to establish a local seed

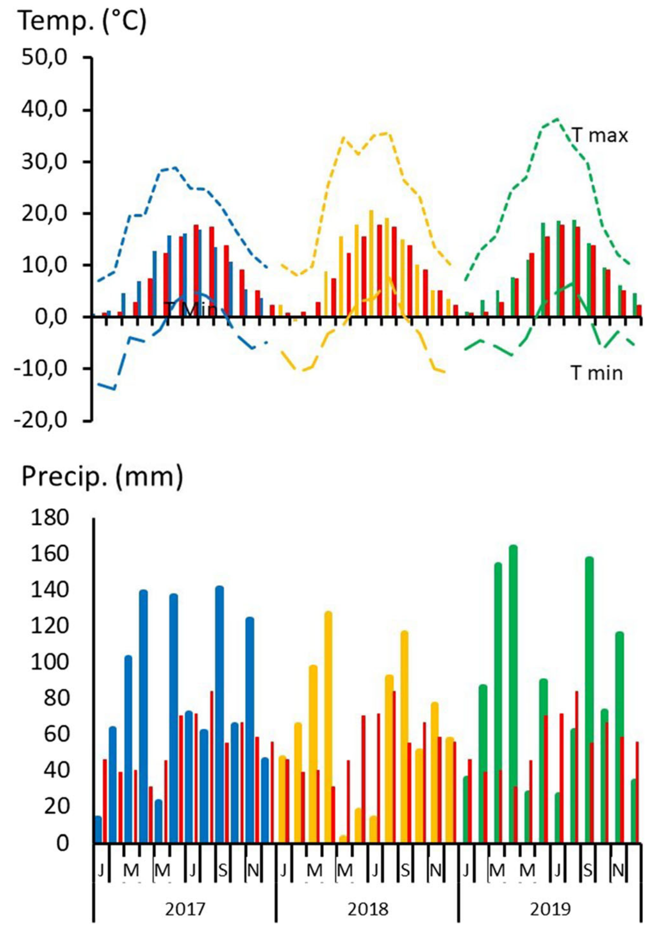

Fig. 3. The monthly mean (bars), monthly minimum (dashed lines), monthly maximum (dotted lines) and 30-year mean temperature (thin bars) temperatures (°C). The monthly (bars) and the 30-year (thin bars) mean precipitations (mm). Colour codes for bars and lines: blue for 2017, yellow for 2018, green for 2019, and for red the 3-year means. Site-specific climate conditions at the SAFE facility are collected by the *in situ* automatic weather station and retrieved from the SITES data portal (https://data.fieldsites.se/portal/). Abbreviations: Temp., temperature; T MEAN, monthly mean temperature; T MIN, monthly minimum temperature; T MAX, monthly maximum temperature; Precip. precipitation.

production plot (5 kg sown on 3000 m^2 in September 2014) for the establishment of the perennial systems in SAFE.

The IWG sole crop and IWG-alfalfa intercrop was sown in May 2016 with complementary sowing in September 2016 (Table 1). Both the sole crop and the intercrop were sown with 25 cm row spacing with alternate rows of IWG and alfalfa in the intercrop (i.e. replacement design). The IWG sowing density was not possible to record, due to faults in the sowing equipment, which also led to the complementary sowing September 2016. The density was estimated to be approximately 17 kg ha^{-1}. The sowing density of alfalfa inoculated with *Sinorhizobium meliloti* was 8 kg ha^{-1}. In 2017, the IWG and IWG-alfalfa intercrop was fertilised using 444 kg Biofer® ha^{-1} year^{-1} (Gyllebo gödning AB, Malmö, Sweden; Biofer is

Table 1 Management activities in the model system for perennial cereal production. Abbreviation *IWG*, intermediate wheatgrass. The asterisk (*) indicates a missing date in the experimental log, and † indicates interrupted work due to heavy rain.

	Month(s), year(s)
Harrowing (approx. 5 cm depth)	5 Apr 2016
Harrowing (approx. 4 cm depth)	19 Apr 2016
Sowing IWG and alfalfa	2–3 May 2016
Complementary sowing IWG	24 May 2016
Topping	25 Jul 2016
Complementary sowing IWG	Sep 2016*
Topping	25 Nov 2016
Fertilisation (Biofer, see text)	4 May 2017
Row cultivation	5 May 2017
Cutting alfalfa (with trimmer)	5 May 2017
Hand harvest, i.e. sampling for analysis	19–20 Sep 2017
Full harvest	13 Nov 2017
Row cultivation (block C)†	26 Apr 2018
Row cultivation (blocks A, B, D)	14–15 May 2018
Fertilisation (digestate, see text)	18 May 2018
Hand harvest, i.e. sampling for analysis	16 Aug 2018
Full harvest	3 Sep 2018
Fertilisation (digestate, see text)	9–10 May 2019
Hand harvest, i.e. sampling for analysis	Sep 2019*
Full harvest	16 Sep 2019

certified for organic farming) (444 kg Biofer correspond to 40 kg N, 12 kg P and 4 kg K ha^{-1} $year^{-1}$). In 2017, row crop cultivator was used for mechanical weeding in the IWG sole crop. The alfalfa in the IWG-alfalfa intercrop was cut in May 2017 to restrict alfalfa from overgrowing the IWG and the alfalfa residues were left to decompose in the rows as a green manure. In 2018 and 2019, 17 tonnes of biogas digestate per $hectare^{-1}$, corresponding to 35-40 kg N ha^{-1}, were applied as fertiliser to the IWG sole crop and intercrop. No weed management substance was applied in 2018 and 2019.

2.2 Sampling and analyses

Aboveground plant material was sampled in four 0.25 m^2 subplots in each experimental plot before harvest in 2017, 2018 and 2019. The grain and biomass yield of IWG, the biomass yield of alfalfa and the biomass of weeds were determined after threshing and drying (65 °C, 48 h). The dry matter harvest index of IWG was calculated as the percentage grain yield of total dry matter, and the nitrogen harvest index was calculated as the percentage grain N accumulation of total N accumulation. The proportion of C and N, and the isotopic composition of ^{13}C and ^{15}N, was analysed on dried (65 °C, 48 h), milled (<1 mm) plant material using Dumas combustion on an elemental analyser (CE 1110, Thermo Electron, Milan, Italy) coupled in continuous flow mode to a Finnigan MAT Delta PLUS isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). The isotopic analysis was done at the Department of Geosciences and Natural Resource Management at the University of Copenhagen.

2.3 Land equivalent ratio

The Land Equivalent Ratio, LER (Willey and Osiru 1972), is the area of legume and cereal monocrops required to produce the same amount of grain as one unit area of the cereal–legume intercrop. Since no monocrop of alfalfa was available, the partial Land Equivalent Ratio of IWG, $p\mathrm{LER}_{\mathrm{IWG}}$, was calculated as the ratio between IWG grain dry matter yield in the monocrop and the intercrop (Eq. 1).

$$pLER_{IWG} = \frac{Y_{IWG\ grain\ of\ monocrop}}{Y_{IWG\ grain\ of\ intercrop}} \quad (1)$$

2.4 Nitrogen fixation and soil N acquisition in alfalfa

The ^{15}N abundance in legume and non-legume samples, expressed as $\delta^{15}N$ (‰ deviation from the ^{15}N abundance in atmospheric N_2; Unkovich et al. 2008), was used to calculate the proportion (%Ndfa; Eq. 2) and the amount (N_{FIX}; Eq. 3) of N in the aboveground legume biomass that was derived from biological N_2 fixation.

$$\%Ndfa = \frac{\delta^{15}N_{reference} - \delta^{15}N_{legume}}{\delta^{15}N_{reference} - B} \times 100 \quad (2)$$

$$N_{FIX}\ \left(\text{kg N ha}^{-1}\right) = N_{YIELD}\ \left(\text{kg N ha}^{-1}\right) \times \frac{\%Ndfa}{100} \quad (3)$$

The mean $\delta^{15}N$ value of the IWG and weed samples from each experimental plot was used as $\delta^{15}N_{\mathrm{reference}}$ to calculate %Ndfa in the legumes present in the same plot. The B value for alfalfa, i.e. the $\delta^{15}N$ in the legume when relying on N_2 fixation as its only N source, was −0.677 (Unkovich et al. 2008), and is included in the equation to account for discrimination against ^{15}N during N_2 fixation and N translocation within the legume plant (Högberg 1997). The values of legume N accumulation (N concentration multiplied by biomass dry weight) and %Ndfa were used to calculate the amount of legume N derived from N_2 fixation (Eq. 3). The amount of N derived from the soil and fertiliser (N_{SOIL}) constitutes the remaining proportion N acquired (Eq. 4).

$$N_{SOIL}\ \left(\text{kg N ha}^{-1}\right) = N_{YIELD}\ \left(\text{kg N ha}^{-1}\right) \times (1 - \%Ndfa) \quad (4)$$

2.5 Discrimination against ^{13}C

Natural abundance of ^{13}C was used to calculate the discrimination against ^{13}C ($\Delta^{13}C$), which is positively related with water use efficiency (Farquhar et al. 1982; Farquhar et al. 1989; Farquhar and Richards 1984; O'Leary 1981). The ^{13}C composition ($\delta^{13}C$) is given by the measured ratio of $^{13}C/^{12}C$ and the Vienna Pee Dee Belemnite reference material, where R_{PDB} = 0.01117960 (Coplen 2011) (Eq. 5) (Farquhar et al. 1982; O'Leary 1981; O'Leary 1988; Park and Epstein 1960). The discrimination against ^{13}C was calculated (Eq. 6), using δ_{atmos} and δ_{plant} which refer to the carbon isotope composition of the atmospheric CO_2 and plant material, respectively. The carbon isotope composition of the atmospheric CO_2, δ_{atmos}, is approximately −8.0‰ (Farquhar et al. 1989; O'Leary 1988).

$$\delta^{13}C_{PDB}\ (‰) = \left(\frac{R_{sample}}{R_{PDB}} - 1\right) \times 1000 \tag{5}$$

$$\Delta^{13}C\ (‰) = \frac{(\delta_{atmos} - \delta_{plant})}{1 + \delta_{atmos}/1000} \tag{6}$$

2.6 Statistics

The effects of intercropping on the grain and straw yields, the N concentrations in dry matter, the N accumulated in the aboveground biomass and the discrimination against 13C were statistically analysed using a factorial design with repeated measures. In the model, block was a random factor and the 'crop', 'year' and the interaction between 'crop' and 'year' were fixed factors with the correlation structure AR(1) for observations from the same block and 'crop'. The analysis was done using proc MIXED in SAS (SAS 9.4) with Kenward-Rogers method for denominator degrees of freedom. Tukey's post hoc test at the $p < 0.05$ level of significance was used for pairwise differences between treatment levels. The interaction 'crop*year' was significant in all analyses except for intermediate wheatgrass straw yield, harvest index and nitrogen concentration in intermediate wheatgrass grain. The amount of N_2 fixation in legumes was analysed with only 'year' as fixed factor. Bivariate parametric Pearson correlation tests, with $p < 0.05$ as the level of significance, were run to explore the relationships between discrimination against ^{13}C, yield, N concentrations and N accumulation. The correlations were run on data separated by year (2017, 2018 and 2019), cropping system (monocrop and intercrop) and yield fractions (grain and straw). The correlations were performed with IBM Statistics SPSS software.

3 Results and discussion

3.1 Dry matter production

The IWG grain dry matter yield varied between 0.26 and 0.88 t ha^{-1} in the monocrop and between 0.28 and 0.55 t ha^{-1} in the intercrop (Fig. 4). The IWG grain dry matter yield was significantly higher in the monocrop than the intercrop in 2017, and significantly greater in 2017 than during the subsequent 2 years in both the monocrop and the intercrop (Fig. 4). The IWG straw biomass did not follow the same pattern as the IWG grain, but was rather stable throughout the 3 years. The IWG straw dry matter yield varied between 5.4 and 8.2 t ha^{-1} in the sole crop and between 4.6 and 5.3 ha^{-1} in the intercrop (Fig. 4). In the IWG monocrop, the IWG straw dry matter yield was significantly lower in the dry year 2018 and then in 2017 and 2019, while in the intercrop, IWG straw biomass was similar over all three experimental years. The alfalfa biomass yield varied between 3.8 and 4.5 t ha^{-1} and did not differ significantly between the experimental years. The IWG grain partial land equivalent ratio in the intercrop was higher in 2018 and 2019 than in 2017 (Table 2). The harvest index (HI) of IWG was highest in both crops in 2017, with declining values over the 3 experimental years (Table 2).

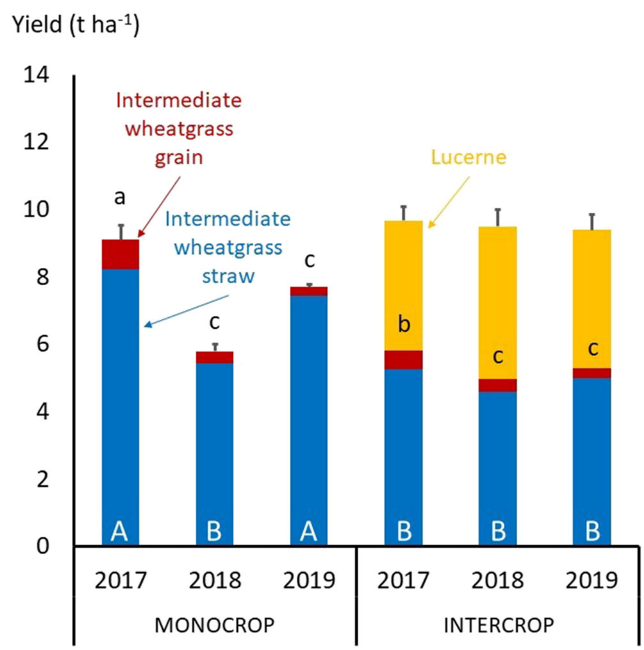

Fig. 4 The aboveground dry matter biomass production of intermediate wheatgrass grain (red bars), intermediate wheatgrass straw (blue bars) and lucerne (yellow bars). Different black lower case letters (above red bars) indicate differences in intermediate wheatgrass grain yield, white upper case letters (within blue bars) indicate differences in intermediate wheatgrass straw yield, from proc MIXED in SAS (SAS 9.4) with Kenward-Rogers method for denominator degrees of freedom, using univariate ANOVA with Tukey's post hoc test at the $p < 0.05$ level of significance.

Table 2 The partial Land Equivalent Ratio for IWG ($pLER_{IWG}$), harvest index (HI, %) and nitrogen harvest index (NHI, %) of IWG presented as means ($N = 4$) with standard errors. Different lowercase letters indicate differences between treatments (crop and year) from proc MIXED in SAS (SAS 9.4) with Kenward-Rogers method for denominator degrees of freedom and Tukey's post hoc test at the $p < 0.05$ level of significance (SAS 9.4). Abbreviation IWG, intermediate wheatgrass.

	$pLER_{IWG}$	*HI* [%]	*NHI* [%]
IWG monocrop			
2017	n. a.	9.60 ± 0.39***a***	23.3 ± 2.4***ab***
2018	n. a.	6.23 ± 0.87***bc***	25.2 ± 2.8***a***
2019	n. a.	3.38 ± 0.20***d***	16.5 ± 2.3***bc***
IWG intercrop			
2017	0.63 ± 0.049***b***	9.55 ± 1.3***a***	14.8 ± 3.0***c***
2018	1.08 ± 0.16***a***	7.80 ± 0.89***ab***	26.3 ± 2.3***a***
2019	1.07 ± 0.11***a***	5.38 ± 1.0***c***	19.8 ± 4.2***abc***

The levels and pattern of the IWG grain yields are in coherence with what other reports (Hunter et al. 2020; Jungers et al. 2019; Tautges et al. 2018), despite the comparably low amount of fertiliser used in our low-input management design. The reported IWG grain dry matter production levels are mainly stemming from the IWG germplasm coming from the TLI third cycle of IWG, which is quite an early line in the IWG breeding programme. The programme is constantly making progress towards higher seed yield (DeHaan et al. 2013) together with other important agronomic features (Wagoner and Schauer 1990). In parallel to a higher grain yield, the longevity of IWG grain production is also questioned. The decline in grain yield over years may be reduced under high fertilisation rates (120–160 kg N ha^{-1}) (Culman et al. 2013; Jungers et al. 2019), but may be simultaneously associated with stronger environmental impacts through leaching and N_2O emissions. However, the large and deep root system of IWG (Sprunger et al. 2019) and the high capability of IWG to exploit the soil volume (Duchene et al. 2020), compared to annual crops, may reduce the leaching risk to a minimum (Culman et al. 2013; Jungers et al. 2019).

The higher IWG grain partial land equivalent ratio in the intercrop in 2018 and 2019, than in 2017, indicates the better growing conditions for IWG when intercropped with alfalfa. The increasing amount of N becoming available from the alfalfa over time, together with a lower density, and thus lower intraspecific competition, of IWG in the intercrop may also positively influence the performance of IWG in the intercrop compared to in the monocrop. The HI follows the pattern of declining grain yield over years, which is in line with the findings from Hunter et al. (2020) who reported that yield of IWG declined, due to low grain number, few highly productive spikes, increased intra-stand competition and declined resource allocation to reproduction over time, motivating future studies focused on maintaining seed set, and thus productivity from a management perspective. The decline in the harvest index was less pronounced in the intercrop as compared to the sole crop, which may be attributed to the expected improved nitrogen supplies provided by the alfalfa root and shoot turnover resulting in a green manure (Bedoussac et al. 2015; Jensen et al. 2020). The growing season of 2018 was unusually dry and warm, resulting in severe drought effects. The hampered growth is not only attributed to physiological constraints, but also to restricted ability of the plant to make use of nutrients, due to lack of precipitation and resulting low soil moisture level. In fact, the digestate applied that year was crusting on the soil surface, seemingly inaccessible to the crops during peak of growth, but later in the season, the nutrients may have become accessible. However, alfalfa did sustain its growth also under the dry conditions in 2018, probably due to its ability to biologically to fix N_2.

3.2 Nitrogen concentration in dry matter and N accumulation

The grain N concentration of IWG varied between 2.7 and 3.5% N. Several intercrop studies of annual cereals and grain legumes have shown that intercropping increases the nitrogen/protein concentration of the intercropped cereal grain and potentially also the baking quality (Gooding et al. 2007; Hauggaard-Nielsen et al. 2008). In 2018, the N concentration of IWG grain is higher when intercropped than when grown as monocrop, while the N concentration in the IWG straw was higher in the intercrop compared to the monocrop in each individual year (Table 3). In the intercrop, the N concentration in IWG straw was higher in 2017 as compared to in 2018 and 2019, while there was no difference between years in the monocrop. The N concentration of the alfalfa biomass was stable at approximately 2.6% N throughout the 3 years (Table 3).

Higher N concentration of the harvested IWG material from intercropped conditions may have positive implications for the use of the IWG straw as forage in animal production as well as the use of the IWG grain in the food industry. Surprisingly, the N concentration in the grain and straw of IWG tends to decline with time both in the sole and the intercrop. Even though just evident as a weak tendency, it can be seen in both the monocrop and the intercrop as well as for both grain and straw components of IWG. This decline may be a result of the low-input management strategy applied to the two crops in this particular experiment. The land of the experimental site was previously used for conventional cropping with a crop rotation based on only annual crops supplied with generous nutrient additions. Thus, the first experimental year is highly influenced by carry-over effects from previous management, while the subsequent years better represent the

Table 3 The nitrogen concentration (N, %) and discrimination against ^{13}C ($\Delta C13$, ‰) presented as means ($N = 4$) with standard errors. Different lowercase letters indicate differences between treatments (crop and year) from proc MIXED in SAS (SAS 9.4) with Kenward-Rogers method for denominator degrees of freedom and Tukey's post hoc test at the $p < 0.05$ level of significance (SAS 9.4). Abbreviations IWG, intermediate wheatgrass; LUC lucerne.

	N [%]			*ΔC13* [‰]	
	IWG grain	IWG straw	LUC total	IWG	LUC
Monocrop					
2017	3.01 ± 0.029***ab***	0.655 ± 0.083***bcd***	n. a.	20.3 ± 0.20***a***	n. a.
2018	2.69 ± 0.060***b***	0.530 ± 0.023 ***cd***	n. a.	18.4 ± 0.20***c***	n. a.
2019	2.66 ± 0.23***b***	0.474 ± 0.037***d***	n. a.	19.0 ± 0.29***b***	n. a.
Intercrop					
2017	3.45 ± 0.057***a***	1.35 ± 0.15***a***	2.46 ± 0.48***a***	20.4 ± 0.12***a***	21.5 ± 0.23***a***
2018	3.39 ± 0.073***a***	0.804 ± 0.032***b***	2.69 ± 0.18***a***	17.6 ± 0.33***d***	19.1 ± 0.20***b***
2019	3.11 ± 0.39***ab***	0.714 ± 0.078***bc***	2.58 ± 0.20***a***	19.1 ± 0.21***b***	22.2 ± 0.17***a***

targeted low-input system with limited nutrient resources. In the light of the multipurpose use of IWG, i.e. both as a grain and feed crop, intercropping is known to improve the nutrient concentration of the biomass and thus its suitability as animal feed (Favre et al. 2019) or biogas digestate usage of the straw fraction. In the context of a changing climate, in terms of the more frequent drought spells, the higher N concentration in

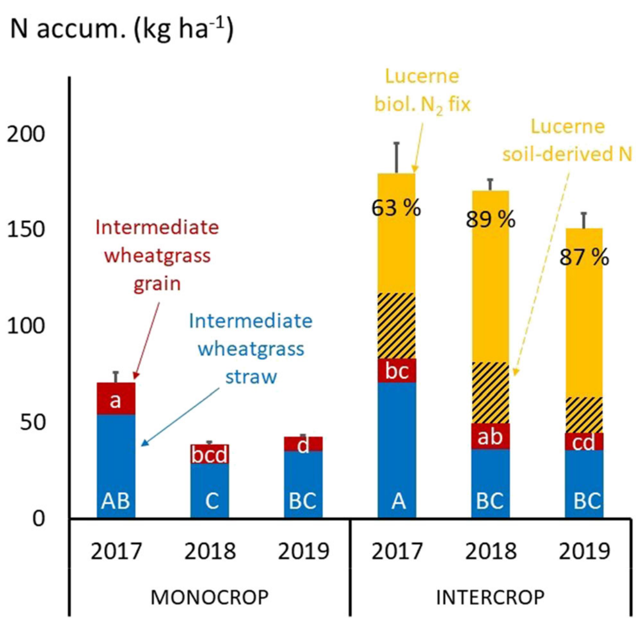

Fig. 5 The aboveground N accumulation in intermediate wheatgrass grain (red bars), intermediate wheatgrass straw (blue bars) and lucerne (yellow bars) divided into the fraction acquired from the soil and fertiliser (black-striped yellow bars) and the contribution of biological N_2 fixation (plain yellow bars). For the latter, the percentage (%Ndfa) of the N_2 fixed is given. Different lower case letters (within red bars) indicate differences in intermediate wheatgrass grain yield, and upper case letters (within blue bars) in intermediate wheatgrass straw biomass yield, from proc MIXED in SAS (SAS 9.4) with Kenward-Rogers method for denominator degrees of freedom, using univariate ANOVA with Tukey's post hoc test at the $p < 0.05$ level of significance.

IWG grain in the dry year of 2018 may indicate a suitable crop for climate adaptation.

The N accumulation (Fig. 5) of IWG grains was higher in 2017 than in 2018 and 2019 in the monocrop. The N accumulated in IWG grains in the intercrop was higher in 2018 than in 2019, while neither differed from 2017. Furthermore, the N accumulated in IWG grains was higher in the monocrop in 2017 than in the intercrop in 2017, while there was no difference between the cropping systems on 2018 and 2019. In the monocrop, the N accumulation of the IWG straw (Fig. 5) was higher in 2017 compared to in 2019. In the intercrop, the N accumulated in IWG straw was higher in 2017 compared to in 2018 and 2019. No differences were found between the two cropping systems within each year. The amount of N accumulated in alfalfa did not differ between years. Neither did the amount of N accumulated from the soil and fertiliser, but the amount of N derived from the atmosphere was higher in 2017 than in the 2 subsequent years (Fig. 5). Our study shows that high amounts of N in lucerne (63 ± 27, 89 ± 2.8 and 87 ± 4.3%; Fig. 5) are derived from the atmosphere and smaller amounts of N (11 to 37%) are derived from soil. The proportion of N_2 fixed from the atmosphere showed a tendency to be higher in 2018 and 2019 compared to in 2017 ($F = 4.69$ (df = 2) $p < 0.1$).

The amount of accumulated N in the studied systems is clearly connected to the amount of biomass produced, further supporting the argument above that growing conditions and production are related to the availability of resources (Hawkesford 2011), in this case nitrogen. The patterns of the N accumulation of IWG grains most probably arise from a depletion of time in the cropping systems, with the pattern more pronounced in the monocrop than in the intercrop (where the depletion is less pronounced) since legumes improve the availability of soil N (Jensen et al. 2020). Interestingly, the rather high level of accumulated N in IWG grain in the intercrop in 2018 potentially demonstrates intercropping as a management tool for production security under unexpected or deviating climate events, such as drought. The lowest values of accumulated N in IWG grains were found in the monocrop in 2019, which may indicate that the IWG crop received a suboptimal supply of N with the low-input management. However, the legume companion is expected to supplement some of the nutrient requirements with time and the tendency of increased fixation of nitrogen from the atmosphere somewhat meets this expectation. This tendency reflects the interspecies interaction in intercrops of cereals and legumes, where the cereal most often acquires a larger proportion of the soil N in comparison to its proportional abundance, which leads to increased N_2 fixation in the grain legume crop (Rodriguez et al. 2021). On the other hand, Li et al. (2019) identified a need for fertilisation to support the development of IWG in the establishment phase when intercropped with alfalfa, possibly indicating that alfalfa is too aggressive for intercropping with IWG, at least when established at the same time point. The study of Li et al. (2019) needs validation in the field, but gives clear indications that we need more knowledge on appropriate intercropping companion and establishment practices for IWG production. Surprisingly, the aboveground N accumulation in IWG straw did not differ between the monocrop and the intercrop in either of the 3 years, despite the IWG straw biomass was generally lower in the intercrop than in the sole crop. Under intercropped conditions as those in this experimental setup, the cereal is established on half of the area compared to that in the monocrop. Thus, our results clearly demonstrate the benefits of intercropping in terms of nutritional value (Favre et al. 2019; Bedoussac et al. 2015), irrespective of the end-usage of the crop.

3.3 Discrimination against ^{13}C as an indicator of water use efficiency

In the dry year of 2018 (Fig. 3), the discrimination against ^{13}C in the IWG aboveground biomass was lower in both the sole cropped and the intercropped system compared to the other years, i.e. 2017 and 2019 (Table 3). This was probably a response to drought, which has been observed in other C_3 forage grasses (Mårtensson et al. 2017), where improved water use efficiency is gained through stomatal closure (Farquhar et al. 1982; Farquhar et al. 1989). Water limitation is often closely connected to lowered availability of nutrients (Kreuzwieser and Gessler 2010) partly through the limitation of microbial activity (Borken and Matzner 2009) and hence nutrient mineralisation, but also through the restricted transport and uptake of soil water and the associated mass flow uptake of nutrients. Water limitation seems to be better met when intercropping with legumes, where IWG showed even lower discrimination against ^{13}C when intercropped with alfalfa. Some varieties of alfalfa have been described as drought resistant (Guo et al. 2005), due to their extensive and deep root system (Dolling et al. 2003; Julier et al. 2017). Indeed, the reduced discrimination under drought also occurred in alfalfa shoots, where the discrimination against ^{13}C was lower in 2018 compared to in 2017 and 2019 but this legume exhibits low stomatal closure in the early stages of drought (Durand 2007). Furthermore, it can be hypothesised that alfalfa potentially provides a shading effect on the soil surface, thus reducing the evaporation and improving the soil water status.

Under intercropped management, discrimination against ^{13}C in IWG grains was well correlated to IWG grain yield ($\rho = 0.971$, $p < 0.05$; data not shown) and IWG grain N accumulation ($\rho = 0.987$, $p < 0.05$; data not shown) in 2018. In 2019, positive correlations were found between discrimination against ^{13}C in IWG straw and both IWG straw yield ($\rho = 0.982$, $p < 0.05$; data not shown) and IWG straw N yield ($\rho = 0.961$, $p < 0.05$; data not shown) in intercrops. These results clearly demonstrate the ability of IWG to improve water use efficiency, which may lead to downregulation of the discrimination against ^{13}C to sustain photosynthesis under the dry summer of 2018 and the possibly still dry soils in 2019. These results are supported by other studies which have proven that IWG maintains relatively high water-use efficiency during the growing season (Culman et al. 2013; de Oliveira et al. 2020), which helps to mitigate water stress.

4 Conclusions

Here, we show for the first time potential benefits of the intercropping of perennial cereal crop, IWG, with alfalfa in terms of grain yield and biomass production in Scandinavia, also under a drought spell. In particular, the ability to acquire N_2 from the atmosphere to the production system under intercropped conditions over the experimental period (2017–2019) illustrates the important function of acquiring additional nitrogen from the atmosphere into this production system, especially under dry conditions to sustain photosynthesis and, thus, growth. We suggest that perennial cereal crops intercropped with legume companions could be a suitable addition to cropping systems under the expected increased frequency of drought events.

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Authors' contributions Jensen was responsible for the development and design of the SAFE. Li carried out sample preparation for laboratory analyses. Dimitrova Mårtensson carried out statistical analyses. Results were interpreted by Dimitrova Mårtensson, Li, Barreiro and Jensen. The manuscript was written by Dimitrova Mårtensson with input from Li, Barreiro and Jensen.

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Data availability The data generated and analysed during this study are available from the corresponding author upon reasonable request.

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Declarations

Ethics approval Not applicable.

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Conflict of interests The authors declare no competing interests.

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