Maintaining grain yield of *Th. intermedium* across stand age through constant spike fertility and spike density: understanding its response to various agronomic managements.

Fagnant L.[1*], Duchene O.[2], Celette F.[2], Dumont B.[1]

Corresponding author: laura.fagnant@uliege.be; +3281622142

[1] ULiege - Gembloux AgroBio-Tech, Plant Sciences Axis, Crop Science lab., B- 5030 Gembloux, Belgium.
benjamin.dumont@uliege.be

[2] ISARA, Agroecology and Environment Research Unit, 23 Rue Jean Baldassini, 69364, Lyon cedex 07, France.
olduchene@isara.fr; fcelette@isara.fr

ABSTRACT

Thinopyrum intermedium subsp. *intermedium* (Host) Barkworth & D.R. Dewey is a perennial grass proposed as a dual-use crop for both forage and grain. Being in the nascent stage of domestication, its grain yield potential is still low compared to annual counterparts. The understanding of *Th. intermedium* development and the resulting grain yield in field is limited along with its response to agronomic management. To identify the interrelations between development traits and their influence on grain yield, various crop measurements were evaluated during four growing seasons in field grown *Th. intermedium* conducted under various autumn defoliation operations and nitrogen (N) fertilizations. Under sufficient N treatments (i.e., 100 kg N ha^{-1}), grain yield remained constant over the four years of the experiment with a mean of 1 t ha^{-1} resulting from a constant spike fertility and a spike density level above 400 spikes m^{-2}. However, significant mortality and inhibition of reproductive growth of tillers can occur when number of tillers is too high and/or resources too scarce (e.g., unfertilized plots or water deficiencies). In addition, excessive aboveground production at the beginning of the reproductive phase can be detrimental to the final grain yield through the negative influence of tiller density and aboveground biomass (DM) on the yield per spike. The highest aboveground production was observed during the second year with 1415 tillers m^{-2} at the beginning of the reproductive phase and 16 t of DM ha^{-1} at grain harvest. Although grain yield response to N fertilization was positively associated to spike density, excessive aboveground biomass could be enhanced by N fertilization. The fertilization of 50 kg ha^{-1} in autumn combined with a fertilization of 50 kg ha^{-1} in early-spring could sustain tiller fertility without hampering grain production. In autumn, plant regrowth was low with the highest value of 1.3 t of DM ha^{-1} observed in the first year. Autumn defoliation could be used to maintain the yield per spike in case of excessive biomass production by the reduction of the DM and the final tiller density coupled with the increase of the grain weight the next year. Finally, shredding post-harvest crop residues at ground level to promote light penetration at the bases of plants may have a positive influence on the tiller fertility. Therefore, under fertile soil conditions (i.e., deep soils and sufficient N fertilization) combined with an optimal stand establishment (i.e., from 500 to 1000 tillers m^{-2} at the beginning of the growing season) we demonstrated that grain yield potential can be maintained as the crop ages. In the future, breeding should raise resource allocation to the grain by increasing yield per spike and avoid overproduction of new tillers through tillering or rhizome propagation without compromising the vigor of regrowth and its environmental performance.

1. Introduction

Thinopyrum intermedium subsp. *intermedium* (Host) Barkworth & D.R. Dewey is a perennial grass, some populations of which have been bred over three decades for use as a perennial grain crop. Such crop is proposed for dual-purpose use, with both forage and grain for human consumption. While grain and straw are harvested in summer, additional cut or grazing operations could be

46 performed in autumn or in early-spring for forage production (Culman *et al.*, 2023; Favre *et al.*, 2019;
47 Hunter *et al.*, 2020b). Grown for several years, the extended belowground activity and the year-
48 round soil cover can provide important benefits for soil protection, carbon storage or water quality
49 (Culman *et al.*, 2013; de Oliveira *et al.*, 2018; Sprunger *et al.*, 2019). Among several perennial grasses,
50 *Th. intermedium* has been preferred due to its agronomic potential for grain production (i.e., initial
51 seed size, free threshing ability, or regrowth vigor) (Wagoner, 1990). Since 2003, improved
52 populations for yield per spike, seed mass and percentage of naked seeds are bred at The Land
53 Institute (Kansas, US), and more recently through other breeding programs in different universities in
54 North America and Sweden (Bajgain *et al.*, 2022). With an estimate of yield increase of 58 kg ha^{-1} per
55 cycle (Bajgain *et al.*, 2022), important progress has been achieved, but *Th. intermedium* is in the
56 nascent stages of domestication and its yield potential is still low compared to annual counterparts
57 reaching a maximum of 10 to 20 % of what best wheat modern lines can achieve in a large range of
58 pedoclimatic conditions (Culman *et al.*, 2013; Larkin *et al.*, 2014; Newell & Hayes, 2017). Due to this
59 lower grain production, its adoption in farming systems should promote its multifunctionality (Ryan
60 *et al.*, 2018; Duchene *et al.*, 2019). Alongside the breeding work, enhancing productivity in the field
61 requires a better understanding of the ecophysiological functioning of the crop in relation to
62 management practices.

63 Grain yield is influenced by multiple component traits themselves explained by a complex underlying
64 genetic architecture and growth conditions. The grain yield of an annual cereal crop can be split into
65 three major components (Hay & Porter, 2006). First, the spike density which is determined primarily
66 by plant population density, but also by tiller initiation and loss. Second, the spike size which is set
67 during spikelet initiation, and whose productivity will ultimately depend on the survival rate of the
68 spikelets and florets, as well as the fertility of the florets. And last, the mean grain weight, not strictly
69 under development control, is determined by the quantity of assimilates available to the spike. In
70 perennial grasses, the dynamics of tiller appearance, developmental differentiation (i.e., vegetative,
71 rhizomatous, or reproductive) and death underlie plant survival over multiple growing seasons
72 (Durand & Lafarge, 2011). Although the developmental pattern and the regulation of tiller
73 emergence have been investigated (Assuero & Tognetti, 2010), the way genetic and environmental
74 factors interact to determine tiller fate and fertility is still largely unclear. In swards, among the
75 important peculiarities of perennial grasses compared to annual cereals, we can identify i) a highly
76 variable and often low proportion of fertile tillers with aging plants, resulting in grain yield decrease
77 over years (Fulkerson, 1980; Jungers *et al.*, 2017), ii) the marked influence of the period of
78 appearance of tiller cohorts and the order of emergence of the tillers on their reproductive potential
79 (Rouet *et al.*, 2021), iii) an increased variability in floret site utilization (Elgersma, 1985; Altendorf *et
80 al.*, 2021) and iv) the potential competition between several sink organs at the end of the growing
81 season (i.e., grains, rhizomes, bulbs, stolons or dormant buds) (Hay & Porter, 2006; Durand &
82 Lafarge, 2011). These characteristics have fueled intense theoretical discussion as to whether
83 physiological trade-offs between plant longevity and seed production can effectively be overcome
84 through new agroecosystem design and modern breeding techniques (see for example DeHaan *et al.*
85 (2023)).

86 The understanding of grain yield build-up of *Th. intermedium* is still limited, but recent studies
87 identified multiple positive correlations between the seed yield and several traits such as
88 aboveground biomass, reproductive tiller density, spike length, plant height or flag leaf area (Zhang
89 *et al.*, 2016; Cattani & Asselin, 2018; DeHaan *et al.*, 2018; Bajgain *et al.*, 2019; Bajgain & Anderson
90 2021). Altendorf *et al.* (2021) suggests that biomass *per se* is a poor indicator of grain yield, while the
91 reproductive tiller density at harvest and floret site utilization (ranging from 12 to 60 %, Larson *et al.*,
92 2019; Altendorf *et al.*, 2021) are the main drivers in spaced plants. However, in denser swards, a
93 strong trade-off has been observed between tiller density and fertility, especially as stand ages
94 (Jungers *et al.*, 2017; Hunter *et al.*, 2020a), so that total densities exceeding roughly 1000 tillers m^{-2}
95 were detrimental to grain yield. Additionally, there is a marked trend of yield decline as stand ages,

leading to a drop in the harvest index as high as 50 % (Culman *et al*., 2023; Duchene *et al*., 2023). Different productivity dynamics have been observed in previous studies, with yields peaking the first or the second year of cultivation before dropping down (Jungers *et al*., 2017; Tautges *et al*., 2018; Bajgain *et al*., 2020; Bergquist *et al*., 2022). These various patterns may be explained by compensatory effects between yield per spike (i.e., grain weight and number) and yield per plant (driven by reproductive tiller number) (Altendorf *et al*., 2021). Several processes have been hypothesized to explain yield decline, including increased competition for light or nutrients with higher tiller density (Jungers *et al*., 2017; Tautges *et al*., 2018), more stress-tolerant growth strategy (Law *et al*., 2020), or resource allocation shifts toward perennating organs (Vico *et al*., 2016). At the present time, the selection process does not detect a strong opposition between the increase in grain yield and the vigor of regrowth. On the contrary, grain yield improvement has been suggested to be feasible without major loss of perennation through extended growing season and enhanced resource availability and use (Vico *et al*., 2016; DeHaan *et al*., 2018).

Beside breeding, Bajgain *et al*. (2022) estimated that 31 % of global yield increase would be linked to better management in fields, as it was seen for wheat. Among this, nitrogen (N) management is one major factor. N availability influences crop yield mostly through its effect upon canopy expansion, survival, and longevity of organs (Satorre & Slafer, 1999; Hay & Porter, 2006). The effect on tiller development, survival and growth in perennial grasses has long been demonstrated (Gislum & Griffith, 2005; Assuero & Tognetti, 2010). When seeking to maximizing grain production, N shortage in autumn or early-spring can be detrimental to yields due to source-limited conditions for tiller development, growth, and spikelet's formation (Langer, 1979; Boelt & Studer, 2010; Reynolds *et al*., 2022). However, the effect of N fertilization for seed-bearing grasses is controversial due to unclear effect on tiller and spike fertility, increased lodging risk or secondary tillering (Langer, 1979; Boelt & Studer, 2010). Some studies have investigated the impact of N fertilization on *Th. intermedium* performance (Jungers *et al*., 2017; Frahm *et al*., 2018; Fagnant *et al*., 2023) and suggested an optimal N fertilization ranging from 61 to 100 kg N ha^{-1}. Defoliation operations can have a major impact on grass productivity as well. Defoliation performed above the apex height is known to preserve the tiller reproductive potential. Meanwhile, defoliation may generate new vegetative tillers and could eventually delay stem elongation or reduce tiller height (Mansat & Pfitzenmeyer, 1966; Gillet, 1973; Rouet *et al*., 2021). With respect to *Th. intermedium,* various management options have been studied to improve grain yield in young stands or to maintain yield in aging stands including crop defoliation in summer (i.e., just after grain harvest), in autumn or in spring, either by grazing (Dick *et al*., 2018) or mowing. As the spring mowing tends to decrease grain yield (Zimbric *et al*., 2021; Culman *et al*., 2023), the summer and autumn mowing can increase grain yield compared to no forage harvesting (Pugliese *et al*., 2019; Culman *et al*., 2023), but without mitigating yield decline with stand age (Hunter *et al*., 2020a).

Additional research devoted to the understanding of the physiological traits of *Th. intermedium* grain yield potential is needed. Our first objective was therefore to assess the different developmental traits influencing grain yield and to elucidate their interrelations. Our second objective was to understand the influence of autumn defoliation and N fertilization on crop growth and yield, that could provide additional support for the design of adapted crop management strategy for *Th. intermedium*.

2. Material and methods

2.1. Experimental design

A field experiment was conducted in the experimental farm of ULiège – Gembloux Agro-Bio Tech, Belgium (50°33'58.7" N, 4°42'22.2" E), during four cropping years. A split-plot randomized complete block design was used, with defoliation as the main-plot treatment and nitrogen (N) fertilization as the split-plot treatment, with four replicates of each treatment. Defoliation factor compared two

treatments: i) no autumn defoliation and ii) autumn defoliation. During autumn mechanical defoliation vegetation was clipped to a height of 7 cm and removed simultaneously. Defoliation was only performed in the autumn (i.e., early November) of the first and third cropping year due to insufficient plant regrowth the other years (Table 1). The N fertilization treatments (ammonium nitrate granular) differed according to the total amount and the timing of application and were modified during the study with only some N treatments present during the four cropping years (Table 1). Each subplot (i.e., split plot of 4 * 8 m) was divided by two to obtain one plot being dedicated to grain yield measurement in summer and the other one to destructive sampling during the growing season. The experiment was sown on September 22nd, 2017, with an interrow spacing of 25cm and a density of 250 seeds m^{-2} leading to a plant density of 176 plants m^{-2} in the first cropping year. Seeds used originate from the fifth breeding cycle achieved at The Land Institute (Kansas, USA). The field is characterized by deep and fertile soil conditions with a clay-loam soil type, and optimal nutrients content with a phosphorus content averaging 0.16 mg g^{-1}, potassium content averaging 0.22 mg g^{-1} and organic carbon content averaging 13 g kg^{-1} over the four years of the experiment. Chemical weeding (i.e., 80 g ha^{-1} of clopyralid, 120 g ha^{-1} of fluoxypyr and 800 g ha^{-1} of MCPA on November 17th, 2017, coupled with 960 l ha^{-1} of bentazon and 30 g ha^{-1} of amidosulfuron on April 18th, 2018) and two mechanical weeding (i.e., interrow hoe weeder in late May 2018) were only performed during the first cropping year. Most post-harvest residues (i.e., straw) were removed manually from the field and the remaining part was shredded at 5 cm from the ground after each grain harvest.

Table 1 : (A) N fertilization treatments described with their timings and amounts (kg ha^{-1}) and (B) defoliation treatments of the experimental site during the four cropping years and the statistical analyses performed on the different treatments.

Management treatments					Cropping year where treatment was studied				Performed statistical analyses
(A) N fertilization									
Treatment number	Total N dose (kg N ha^{-1})	Splitting (kg N ha^{-1})			2018	2019	2020	2021	
		Early-spring*	Mid-spring*	Autumn vegetative stage					
1	0	0	0	0	x	x	x	x	ANOVA & path analysis
2	50	50	0	0	x	x	x	x	
3	100	50	0	50	x	x	x	x	
4	100	100	0	0	x	x	x	x	
5	150	100	0	50		x			
6	150	100	50	0			x	x	Path analysis
7	100	0	100	0	x		x	x	
8	150	50	50	50			x	x	
(B) Defoliation									
Summer straw harvest + autumn defoliation						x		x	ANOVA & path analysis
Summer straw harvest (no autumn defoliation)					x	x	x	x	

*The early-spring fertilization was applied just before the reproductive phase (i.e., BBCH29) and the mid-spring fertilization was applied at flowering (i.e., BBCH65) in 2018 and at flag leaf just visible stage (i.e., BBCH37) in 2020 and 2021.

2.2. Data collection

Data were collected during the four cropping years of the experiment (representing four grain production years with a first establishment year and three successive regrowing years). Aboveground biomass was sampled with a 50 x 50 cm quadrat per plot, cut at 5 cm above soil surface, oven-dried (72 h at 60 °C) and weighted to obtain dry matter (DM). Samples were collected at five different main phenological stages during the growing season; four reproductive stages rated with the BBCH scale (Meier, 2018), namely the stem elongation (BBCH30), the flag leaf (BBCH39), the flowering (BBCH65) and the grain maturity (BBCH89) stages as well as a vegetative stage during the autumn regrowth. Spikes, when present, were always separated from the remaining aboveground biomass (i.e., cut just

175   below the lowest spikelet) to be weighted. When collecting total aboveground biomass, the number
176   of tillers and spikes during the reproductive phase (i.e., from BBCH30 to BBCH89 stage) were counted
177   within the quadrat and tiller fertility were calculated by dividing the number of spikes by the number
178   of tillers at BBCH89 stage. The tiller mortality was also calculated by dividing the difference between
179   the number of tillers at BBCH30 and BBCH89 stage by the number of tillers at BBCH30 stage.

180   At grain harvest, plots were combined with a trial combine harvester to obtain the grain yield on a
181   cleaned, but unsorted seeds basis (i.e., a mix of hulled and dehulled seeds). Few days before grain
182   harvest, plant height was measured randomly by selecting five plants from each plot and measuring
183   them from the ground to the end of the spike. On the same five random plants, the length of the
184   spike was simultaneously measured. Thousand kernel weight was determined on each plot using four
185   replicates by counting 100 seeds with a seed counter and then weighting them. Thousand kernel
186   weight was measured on a bare seed basis (TKW) as well as on unsorted seeds (TKW_{un}). TKW_{un} was
187   then used to calculate spike fertility and grain density using Equation 1 and 2 (Hay & Porter, 2006).

188    $$spike\ fertility = \frac{grain\ yield}{spike\ density.\frac{TKW_{un}}{1000}}\ \text{(Equation 1)}$$

189    $$grain\ density = spike\ fertility\ .\ spike\ density\ \text{(Equation 2)}$$

190   With spike fertility in grains spike^{-1}, grain yield in g m^{-2}, TKW_{un} in g and spike density in spikes m^{-2}.

191   Harvest index was calculated by dividing the weight of seeds on a dry matter basis by the weight of
192   total DM at grain harvest (i.e., BBCH89). The nitrogen harvest index was estimated by dividing the N
193   uptake (i.e., N content of the organ multiplied by its dry matter) of grains by the N uptake of the total
194   DM at BBCH89 stage. The N concentration of bare seed and total DM was measured through the
195   Dumas method (Dumas, 1831). Harvest index of spikes was calculated by dividing the weight of seeds
196   by the weight of spikes at BBCH89 stage on a dry matter basis. Fruiting efficiency was calculated by
197   dividing the grain density by the biomass of spike at flowering (in g m^{-2}).

198   According to Jungers *et al*. (2017), estimation of lodging when present was evaluated on a 10-point
199   scale based on both the stem inclination angle and the proportion of the plants that were lodging in
200   the plot. A lodging score of 10 indicated no lodging and a score of 0 indicated severe lodging (i.e., 100
201   % of the plants lodging with stems horizontal to the soil surface). Lodging scores were always
202   determined by the same observer.

2.3. Weather conditions

204   Weather indicators were calculated to characterize the different cropping years. They were
205   calculated for the different development phases: vegetative and reproductive phase. The vegetative
206   phase started from the seeding of the crop until the beginning of stem elongation (i.e., BBCH30) for
207   the establishment year. The vegetative phase of the regrowing years was split in 2 periods: i) from
208   grain harvest (i.e., BBCH89) until the estimation of total DM at the autumn regrowth and ii) from the
209   estimation of total DM at the autumn regrowth until BBCH30 stage. The reproductive phase was
210   divided in 3 main periods to best match crop development: i) a first period starting from BBCH30 and
211   ending at flag leaf stage (i.e., BBCH39), ii) a second period from BBCH39 until flowering (i.e., BBCH65)
212   and ii) a third period from BBCH65 until BBCH89 stage.

213   During the vegetative and reproductive phases 5 main indicators were calculated: the amount of
214   rainfall, the number of dry days (i.e., where the rainfall was lower than 0.6 mm), the number of
215   scalding days (i.e., where the maximum temperature was above 30 °C), the accumulation of solar
216   radiation, the accumulation of growing degree days as performed in Duchene *et al*. (2021) and finally
217   the water balance (Ks coefficient). Water balances were drawn up to assess soil water availability and
218   to estimate the daily depletion of the extractable soil water (TAW) to a depth of 2 m as performed by
219   Duchene (2020). The water balance was calculated from January 31, 2017 (considering a complete

220 filling of the TAW) to estimate the soil water status at the beginning of the establishment year. The
221 daily evolution of the TAW until the end of the experiment was calculated and then determined as a
222 proportion of the total readily available water to calculate the stress indicator (Ks). Equations are
223 presented in the work of Duchene (2020). The various weather indicators are presented in Table 2.

224 *Table 2 : Weather indicators of the experimental site during the four cropping years for the different development phases.*
225 *(A) the first vegetative phase from the seeding or from the estimation of total DM at autumn regrowth until BBCH30 stage.*
226 *(B) the first reproductive phase from BBCH30 to BBCH39 stage, (C) the second reproductive phase from BBCH39 to BBCH65*
227 *stage, (D) the last reproductive phase from BBCH65 to BBCH89 stage and (E) the last vegetative phase from BBCH89 until*
228 *the estimation of total DM at autumn regrowth.*

	Total rainfall (mm)	Number of dry days	Number of scalding days	Total radiation (J cm^{-2})	Growing degree days	Ks coefficient
(A) Sowing or autumn - BBCH30						
[20-09-17 -> 30-04-18]	410	122		137,628	1567	1.00
[08-11-18 -> 26-04-19]	301	106		108,934	1054	0.71
[06-11-19 -> 25-04-20]	305	105		122,341	1135	0.95
[18-11-20 -> 26-04-21]	300	97		111,805	845	0.96
(B) BBCH30 - BBCH39						
[30-04-18 -> 22-05-18]	21	17		45,333	298	1.00
[26-04-19 -> 23-05-19]	48	14		44,741	265	0.91
[25-04-20 -> 24-05-20]	19	21		62,153	351	0.77
[26-04-21 -> 04-06-21]	80	21		70,675	442	1.00
(C) BBCH39 - BBCH65						
[22-05-18 -> 25-06-18]	15	32		63,822	298	0.87
[23-05-19 -> 24-06-19]	83	19		63,780	265	0.72
[24-05-20 -> 24-06-20]	51	22		68,227	351	0.15
[04-06-21 -> 02-07-21]	93	18		55,907	442	0.92
(D) BBCH65 - BBCH89						
[25-06-18 -> 07-08-18]	3	43	9	105,558	946	0.11
[24-06-19 -> 11-08-19]	31	44	7	98,244	946	0.16
[24-06-20 -> 10-08-20]	47	38	6	98,574	900	0.01
[02-07-21 -> 03-09-21]	266	35	0	98,605	1080	1.00
(E) BBCH89 - autumn						
[07-08-18 -> 08-11-18]	121	76		108,718	1330	0.06
[11-08-19 -> 06-11-19]	192	52		91,282	1235	0.21
[10-08-20 -> 18-11-20]	203	62		97,193	1440	0.35
[03-09-21 -> 11-10-21]	23	22		43,383	441	1.00

229 ## 2.4. Data analyses

230 All data analyses were conducted in the R program version 4.1.0 (R Core Team, 2021).

231 ### 2.4.1. Standard statistical analysis

232 N fertilizations were modified during the study, with only some treatments present in all four
233 cropping years (i.e., treatment 1, 2, 3 and 4) and the defoliation was only performed in the autumn
234 of 2018 and 2020 (Table 1). Data subsets were used to performed ANOVA as described in Table 1 and
235 Table 3. Within the different ANOVA conducted, mixed models were used with *lmer* function from
236 the *lme4* package. A two-way ANOVA was first performed where fixed factors were constituted of

the N fertilization and the defoliation. A second two-way ANOVA was performed on the autumn non-defoliated treatments, with the N fertilization and stand age as fixed factors. As tiller density and total DM were measured at different phenological stages during the different cropping years a third fixed factor (i.e., the phenological stage) was added on these two mixed models. When an interaction was observed between factors, ANOVA were performed on the different treatments of the various fixed factors. Within the different mixed models, block, main plot and year, when year was not included as fixed effect through stand age, were considered as random effects. Models were evaluated to ensure they met the assumptions of independence and normality of residuals through the *plotresid* function. Following ANOVA analysis, pairwise comparisons among treatment means were evaluated with the *emmeans* function from the *emmeans* package with a Tukey adjustment for multiple comparisons. Statistical significance was set at 0.05. Grain yield, spike density, tiller fertility, TKW, spike fertility, grain density, spike length, plant height, N content in grains, harvest index, nitrogen harvest index, spike harvest index, fruiting efficiency, tiller density at BBCH30 and BBCH89, tiller mortality, spike DM at BBCH65 and BBCH89, lodging and total DM from BBCH30 to BBCH89 and at the autumn regrowth were the analyzed variables.

2.4.2. Correlation analysis

To evaluate the correlations between crop measurements, the *cor.test* function from the *psych* package was used. Grain yield, spike density, tiller fertility, TKW, spike fertility, grain density, spike length, plant height, harvest index, nitrogen harvest index, tiller density at BBCH30 and BBCH89, spike DM at BBCH65 and BBCH89 and total DM from BBCH30 to BBCH89 and at the autumn regrowth and lodging were the analyzed variables. Verifications of the assumptions of independence (Durbin Watson test), normality (Shapiro test) and homogeneity (Breusch-Pagan test) of the residues were processed. Finally, the non-parametric spearman's correlation coefficient (r) of these different relations was achieved as well as their significant levels, as normality was not always respected.

2.4.3 Path analysis

To assess the direct and indirect effects of crop measurements on grain yield, path analysis was performed. The *sem* function from the *lavaan* package was used on standardized variables (i.e., centered mean and scaled by standard deviation). Only observed variables with a complete set of measurements (i.e., 4 years of data) were included in the path analysis.

2.4.3.1. Development of the initial model

The initial model described in Figure 1 was designed with the assumption and understanding that yield components develop sequentially, and that their effects on one another are unidirectional (i.e., represented by the sense of the arrow; Figure 1), meaning that later developing components do not influence earlier developing components. Vegetative and maturity components are considered as exogeneous variables with no paths leading to them (Figure 1).

The initial model was constructed based on the available literature on *Th. intermedium* and on other grasses and grain crops. Regarding the yield per spike, spike fertility is shown as a function of spike length (Figure 1). Although highly variable, spike length of *Th. intermedium* was shown to be positively correlated with grain yield (Bajgain *et al*., 2019). TKW is a function of grain density (Figure 1), negative relationship between grain weight and grain density have been observed in previous studies on wheat (Slafer *et al*., 2022). Negative correlations were found between seed size and spikelets per spike or spikelet density in *Th. intermedium* (Bajgain *et al*., 2019; Altendorf *et al*., 2021). Concerning vegetative components (i.e., total DM, tiller density and plant height), they are defined to influence grain yield and its components (Figure 1). Indeed, the stem can be at play for translocation to grains during seed fill has shown in perennial ryegrass (Altendorf *et al*., 2021). In addition, plant

height and total DM were shown to be positively correlated with grain yield in *Th. intermedium* (Zhang *et al.*, 2016). Alternatively, grain yield decline in *Th. intermedium* stands over time could be due to the increase of tiller density that causes intraspecific competition and reduces seed production (Jungers *et al.*, 2017) with a negative influence of tiller density and total DM on spike fertility (Hunter *et al.*, 2020a). Finally, in wheat, grain density was related to the amount of resources allocated to the growing spikes (Altendorf *et al.*, 2021; Slafer *et al.*, 2022).

2.4.3.2. Model fitting

To assess that the model fits the data, Chi-square test (χ^2) was used, where an insignificant p-value (p-value>0.05) indicates an acceptable model. Multiple alternative indices were also used; the comparative fit index (CFI) with value above 0.90, the Tucker-Lewis index (TLI) with value above 0.95, the root means square error approximation (RMSEA) as well as the standardized root mean square residual (SRMR) with value below 0.08 are generally indicating a good model fit.
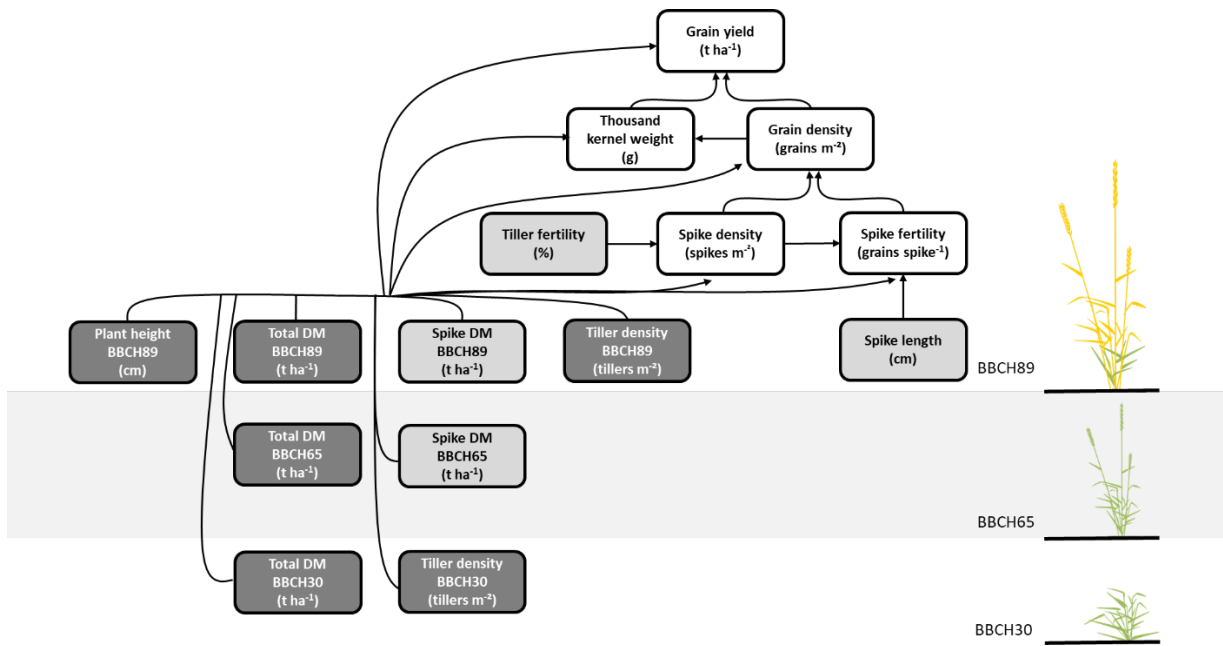
Figure 1 : Initial structural equation model for the relationship between grain yield and crop measurements, followed by their units in parentheses. Groups of traits are colored in different shades of gray, including grain yield and its components (in white), maturity traits (in medium gray) and vegetative traits (in dark gray). A direct path is represented by a single arrow that directly connects two traits (e.g., grain yield and grain density), whereas an indirect path is when the path between a pair of traits is separated by another trait (e.g., grain yield and spike density).

3. Results

Variables	(A) Two-way ANOVA Defoliation (D)	N fertilization (N)	D x N	(B) Two-way ANOVA N fertilization (N)	Stand Age (S)	N x S
Grain yield				***	***	***
Spike density					***	**
Tiller fertility				*	***	
TKW	***				***	
Spike fertility					**	
Grain density					***	**
Spike DM BBCH65		***		***	***	
Spike DM BBCH89					***	
Grain N content	/			***	***	
Spike length				*	***	
Tiller density BCBH30		*			***	
Tiller density BBCH89	*				**	**
Tiller mortality					***	
Plant height		***		***	***	
Total DM BBCH30	***	***		***	***	
Total DM BBCH39		***		***	***	
Total DM BBCH65	**	***		***	***	
Total DM BBCH89				**	***	
Total DM autumn	/			**	***	
Fruiting efficiency		***		***	***	
Spike Harvest index				*	***	
Harvest index				*	***	
Nitrogen harvest index	/				***	
Lodging		***		***	***	**

Note. "*" indicating statistical significance at *p-value*≤0.05; "**" indicating statistical significance at *p-value*≤0.01; "***" indicating statistical significance at *p-value*≤0.001. "/" indicating that the variable was not tested in the ANOVA.

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3.1. Aboveground development

3.1.1. Impact of stand age and phenological stage

As shown in Figure 2, total aboveground biomass varied considerably across stand age (Table 3B). We observed the highest level of total DM during the second reproductive phase (i.e., 2019), reaching a mean of 15.8 ± 0.6 t of DM ha^{-1} at grain maturity stage (i.e., BBCH89). The three other reproductive phases (i.e., 2018, 2020 and 2021) presented similar levels of total DM at BBCH89 stage ranging from 5.7 ± 1.2 for the N-control treatment (i.e., treatment 1) to 10.5 ± 1.5 t of DM ha^{-1} for the N-sufficient treatment (i.e., treatment 4 that is sufficient to achieve a N non-limited crop development see Fagnant et al., (2023)). Total DM produced during the autumn regrowth was low and also significantly influenced by the stand age (Table 3B), with the highest level observed after the first grain harvest (e.g., mean of 1.0 ± 0.1 t of DM ha^{-1} in 2018 compared to 0.2 ± 0.1 t ha^{-1} the other years for the N-control treatment; Figure 2).

Tiller density during the different reproductive phases is presented in Figure 3. Tiller density at the beginning of stem elongation (i.e., BBCH30) varied significantly across stand age (Table 3B), with the lowest level observed during the first year (i.e., 2018, with 463 ± 52 tillers m^{-2} on average) and the highest during the second year (i.e., 2019, with 1415 ± 48 tillers m^{-2} on average). By contrast, tiller density at BBCH89 stage was globally not influenced by the stand age (Table 3B). An exception can be mentioned with the N-control treatment where the highest tiller density at BBCH89 stage was observed in 2019 (p-value<0.001) due to particularly low tiller mortality (i.e., the decrease of tiller density during the reproductive phase; Figure 3). Tiller mortality was the highest during the second year and the lowest during the first year. As opposed to the other years, tiller mortality was not observed in the first year as tiller density increased from BBCH30 to BBCH89 stages (Figure 3; Table 3B).

Plant height was significantly impacted by stand age (Table 3B). The tallest plants were observed in the second year (i.e., 2019 with a mean of 173 ± 2 cm) and the lowest were observed in 2020 (i.e., 125 ± 2 cm on average), marginal means are presented in supplementary material (Table S1).

3.1.2. Impact of N fertilization

Total aboveground biomass varied considerably across N fertilizations (Table 3B). Globally, the N-control treatment had always the lowest total DM and moderate early-spring fertilization (i.e., treatment 2) appeared inadequate to increase total DM during the reproductive phase contrary to treatment 3 and 4 that had the highest total DM (Figure 2). Total DM produced in autumn was positively influenced by the autumn fertilization (i.e., treatment 3) with an increase of 0.3 t ha^{-1} compared to the N-control treatment (Table 3B; Figure 2).

On the contrary, N fertilization had no influence on tiller density (Table 3B). An exception was observed during the first year where lower tiller density at the BBCH89 stage was observed for the N-control treatment (p-value<0.05; Figure 3).

N fertilization had a significant impact on the height of plants (Table 3B). Globally the tallest plants were obtained with the sufficient N treatments (i.e., treatment 3 and 4; Table S1).

3.1.3. Impact of defoliation

Defoliation was performed in the autumn of 2018 and 2020, its influence was therefore only compared in the growing season of 2019 and 2021. Autumn defoliation globally reduced total DM the following year (Figure 4), even if the effect was only significant at the beginning of stem elongation and flowering (i.e., BBCH30 and BBCH65 stages) (Table 3A). Autumn defoliation also reduced tiller density at grain maturity (i.e., BBCH89) of 70 tillers m^{-2} (Table 3A).

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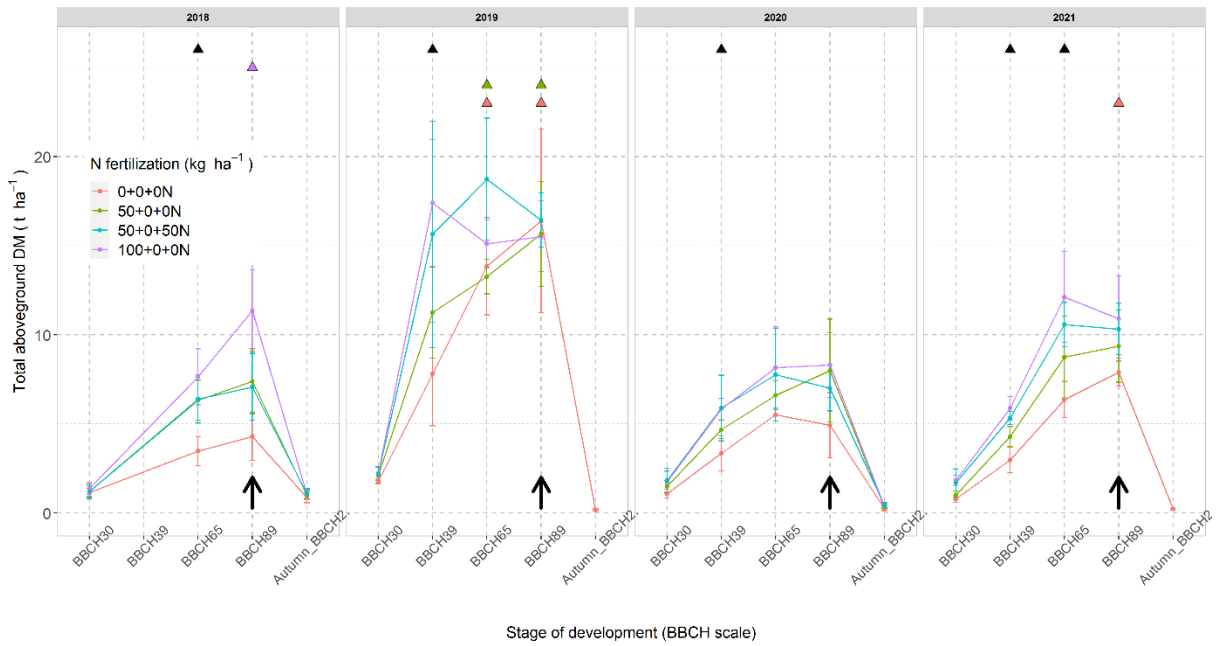
Figure 2 : Mean dynamic of total aboveground biomass during the growing season from 2018 to 2021 on the autumn non-defoliated treatment. Standard errors are represented by error bars. Statistical differences (p-value<0.05; post hoc analysis) between phenological stages are indicated by Δ specifying an increased value; and ∇ specifying a decreased value compared to the previous phenological stage. The color of the symbols represents the N treatment concerned and symbols in black represents the same trends for all N treatments. Arrows is specifying the time of grain harvest with the exportation of straw.

355
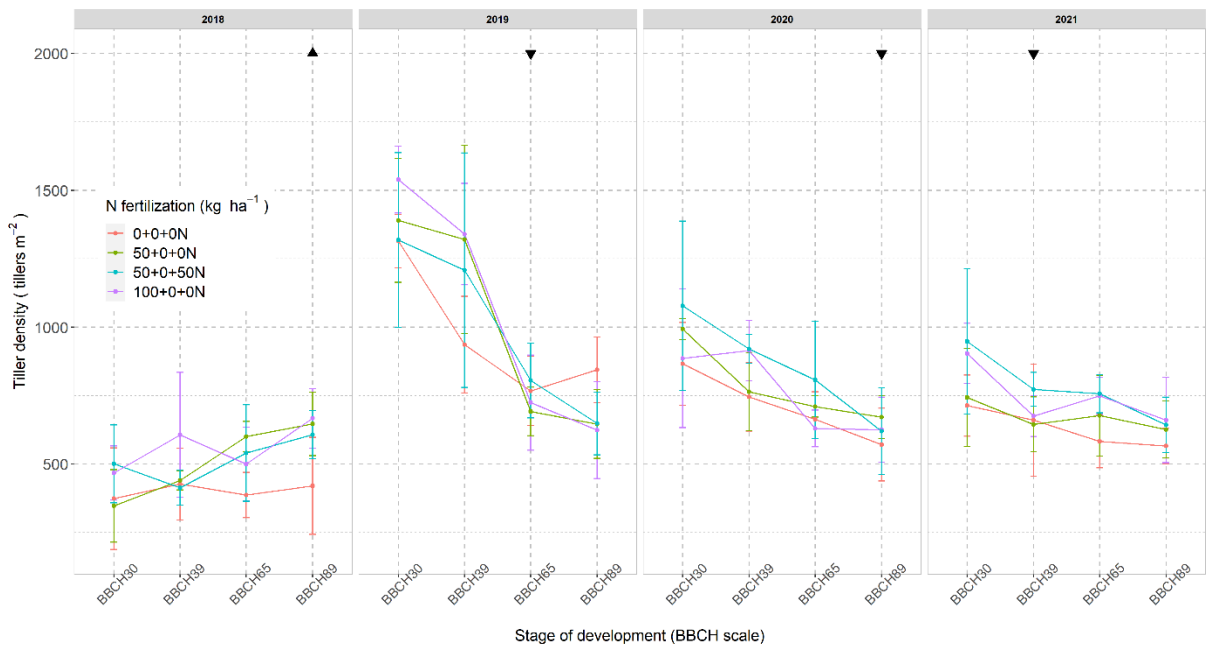
Figure 3: Mean dynamic of tiller density during the reproductive phase from 2018 to 2021 on the autumn non-defoliated treatment. Standard errors are represented by error bars. Statistical differences (p-value<0.05; post hoc analysis) between phenological stages are indicated by Δ specifying an increased value; and ∇ specifying a decreased value compared to the previous phenological stage. The color of the symbols represents the N treatment concerned and symbols in black represents the same trends for all N treatments.
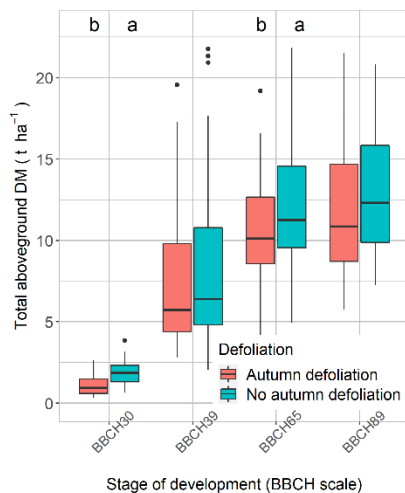
Figure 4 : Effect of autumn defoliation on the total aboveground biomass during the reproductive phase of 2019 and 2021. Letters represent the results of the post hoc analysis (p-value<0.05) of the effect of autumn defoliation on each phenological stage.

3.1.4. Relationship between aboveground development traits

During the growing season, total DM produced at a given stage had a positive influence on the total DM and tiller density measured at next stages (p-value<0.001). Overall, the spike DM was always positively correlated with total DM and tiller density during the growing season as well as with plant height (p-value<0.05), with the strongest correlations found between spike DM and total DM at the same stage (p-value<0.001). However, total DM at autumn seemed negatively influenced by previous aboveground plant development through a negative correlation with the total DM produced at flowering (i.e., BBCH65; p-value<0.001) and with the tiller density during the reproductive phase (p-value<0.01). Correlation coefficients are presented in supplementary material (Table S2).

3.2. Grain production

3.2.1. Impact of stand age

Grain yield, spike density, grain density, spike fertility, thousand kernel weight (TKW) and grain N content through the different stand ages are presented in Figure 5 and marginal means of spike DM, tiller fertility and spike length are provided in supplementary material (Table S1). According to the N treatment, stand age had a different impact on grain yield, spike density and grain density (Table 3B). For N-low treatments (i.e., treatment 1 and 2), its effect was significant (p-value<0.001) with the highest values observed in 2019 and 2021. The lowest grain yield, spike density and grain density were observed in 2018 and 2020 (p-value<0.001) with means of 0.6 ± 0.1 t ha^{-1}, 204 ± 71 spikes m^{-2} and 6482 ± 889 grains m^{-2}, respectively for the N-control treatment. In contrast, N-sufficient treatments (i.e., treatment 3 and/or 4) permitted to maintain constant yields and some components during the four cropping years with means of 0.9 ± 0.1 t ha^{-1} for grain yield, 485 ± 65 spikes m^{-2} for spike density and 11527 ± 1067 grains m^{-2} for grain density (p-value>0.05; Figure 5). Regarding the other parameters, the effect of the stand age was independent of the N fertilization and had a significant impact on tiller fertility, spike fertility, TKW, spike length, spike DM and N content in grains (Table 3B). In the first year (i.e., 2018), TKW and the N content in grains were the highest with a mean of 9 ± 0.2 g for TKW as spike fertility was the lowest (Figure 5). In 2019, we observed the highest spike DM and spike length and the lowest TKW with a mean of 7 ± 0.2 g (Table S1; Figure 5). In 2020, tiller fertility was the lowest with a mean of 56 ± 4 % compared to 74 ± 4 % the other years as spike fertility was the highest with a mean of 31 ± 2 grains spike^{-1} (Figure 5; Table S1).

3.2.2. Impact of N fertilization

The effect of N fertilization was dependent on the stand age for grain yield, spike density and grain density (Table 3B). Concerning grain yield and grain density this effect was significant in 2018, 2019 and 2020 with contrasting results. The effect of N fertilization was positive in 2018 and 2020 and negative in 2019. Contrary to 2018 and 2020, a higher grain yield and grain density was observed in 2019 with the N-control treatment (p-value<0.05; Figure 5). Spike density was positively influenced by N fertilization in 2018 and 2021 (p-value<0.01; Figure 5). Globally tiller fertility, spike length, spike biomass and N content in grains had reduced level with the N-control treatment contrary to the N-sufficient treatments (i.e., treatment 3 and 4) that increased their levels (Table 3B; Table S1; Figure 5). Spike fertility and TKW were not influenced by N fertilization (Table 3B)

3.2.3. Impact of defoliation

Defoliation was only compared in 2019 and 2021, the N content in grains was not measured between defoliation treatments. TKW was the only grain yield components influenced by defoliation in autumn with an increase of 1 g when an autumn defoliation was performed (Table 3A).
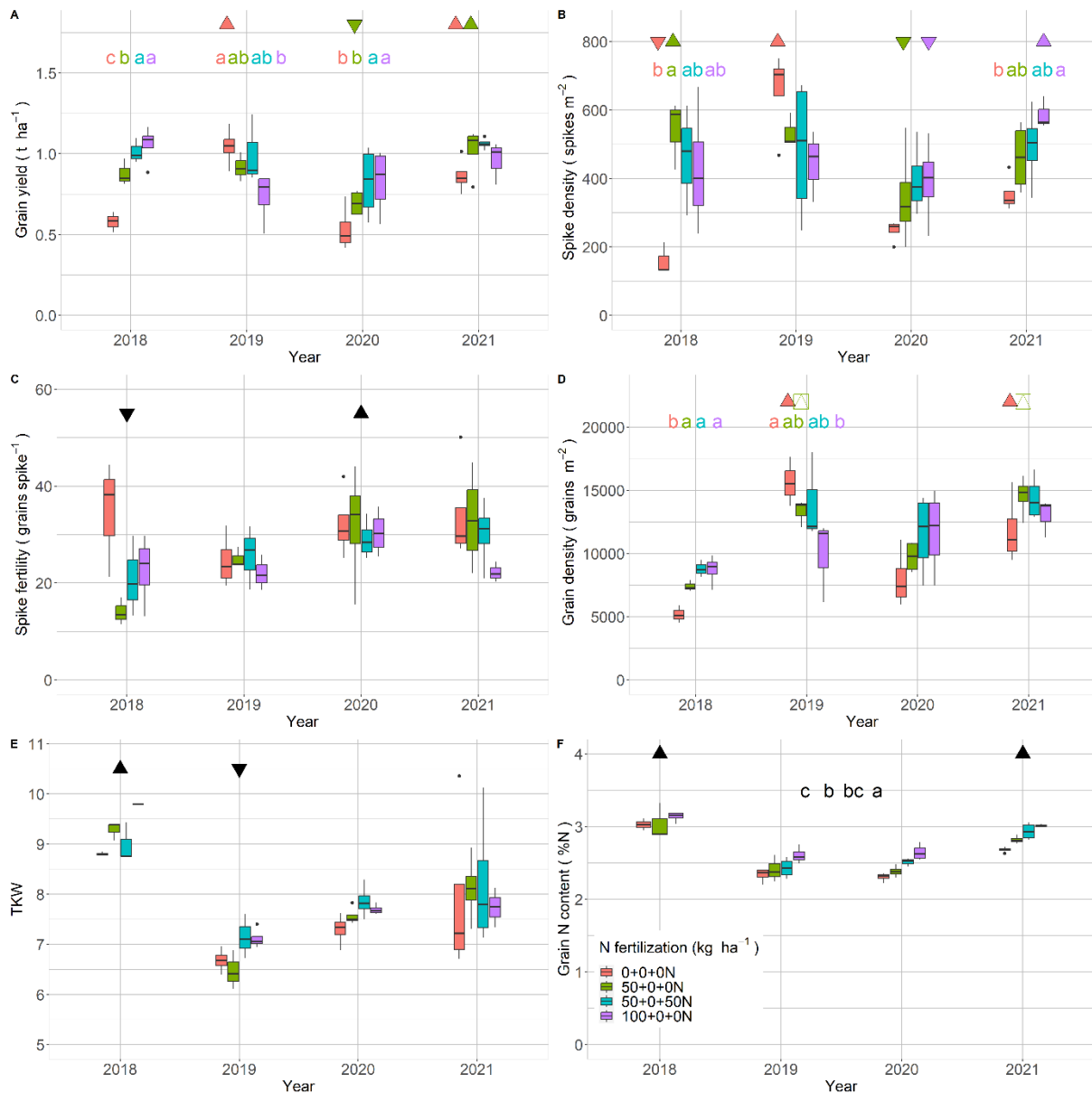
Figure 5 : Effect of N fertilization and stand age on grain yield, its components and grain N content on the autumn non-defoliated treatment from 2018 to 2021. Letters represents the results of the post hoc analysis (p-value<0.05) of the effect of N fertilization and symbols represents the results of the post hoc analysis (p-value<0.05) of the effect of the stand age. Δ specifying an increased value; ⊠ specifying an equal increased value; ∇ specifying a decreased value compared to the other cropping years. The color of the symbols and letters represents the N treatment concerned when there is an interaction between stand age and N fertilization, otherwise the symbols and letters are in black.

3.2.4. Partitioning to reproductive organs

Marginal means of spike harvest index, fruiting efficiency, harvest index and nitrogen harvest index are presented in supplementary material (Table S1). These 4 indicators were influenced by the stand age (Table 3B). Spike harvest index, harvest index and nitrogen harvest index were reduced in the second year (i.e., 2019) with a mean of 45 ± 9 %, 6 ± 1 % and 31 ± 5 %, respectively. The other years, they averaged 72 ± 9 % for spike harvest index, 11 ± 1 % for harvest index and 55 ± 5 % for nitrogen harvest index. Concerning fruiting efficiency, the highest values were observed in 2020 and 2021 with a mean of 143 ± 10 grains g⁻¹ (Table S1). N fertilization had an impact on the spike harvest index, harvest index and fruiting efficiency. The N-control treatment maximized these three parameters as treatment 4 minimized the spike harvest index and harvest index (Table 3; Table S1). Defoliation had no impact on these parameters (Table 3A).

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426 ### 3.2.5. Lodging

427 Lodging was observed in 2019 and 2021, just before the flowering stage (i.e., BBCH65 stage). N
428 fertilization increased lodging severity (Table 3B) where the N-sufficient treatments (i.e., treatment 3
429 and 4) presented the highest lodging severity and the N-control treatment the lowest. Defoliation
430 and stand age had no significant impact on lodging (Table 3).

431 Lodging severity was negatively correlated with grain yield (p-value<0.05), harvest index (p-
432 value<0.01), nitrogen harvest index (p-value<0.05) and fruiting efficiency (p-value<0.001). This could
433 be explained by the negative impact of lodging on the spike fertility (p-value<0.05), the only grain
434 yield component impacted by the lodging in our study. Lodging severity was positively correlated
435 with many indicators of plant development, including tiller density at the beginning of stem
436 elongation (i.e., BBCH30; p-value<0.001), plant height (p-value<0.05) and total DM at previous
437 autumn (p-value<0.05), at beginning of stem elongation, at flag leaf and flowering (p-value<0.001)
438 and at grain maturity (p-value<0.01). Lodging severity didn't have a negative effect on total DM
439 produced the next autumn as the correlation was even positive (p-value<0.05). Correlation
440 coefficients are presented in supplementary material (Table S3).

441 ## 3.3. Grain yield elaboration

442 Acceptable fit indices (see section 'Model fitting' of the material and methods) were observed for
443 path analysis that confirms that the specified model fitted well the data. Fit indices and the variance
444 explained are shown in Table 4. The path coefficients and their significance are shown in Figure 6.
445 Path coefficients are most useful for the comparison of the relative strength of paths within a given
446 path diagram.

447 The direct (σ_{direct}) and indirect ($\sigma_{indirect}$) effect of crop measurements on grain yield are synthesized in
448 supplementary material (Table S4). Grain density was the most positively contributing factor to grain
449 yield with path coefficient σ_{direct} = 0.8 while σ_{direct} of TKW on grain yield was only of 0.1. Spike fertility
450 and spike density had an indirect positive effect on grain yield via grain density ($\sigma_{indirect}$ = 0.7). Tiller
451 fertility and tiller density at grain maturity (i.e., BBCH89) had also a positive indirect effect on grain
452 yield via the increase of spike density and therefore grain density ($\sigma_{indirect}$ = 0.5). Spike DM at
453 flowering (i.e., BBCH65) had a small indirect effect on grain yield by an increase of grain density
454 ($\sigma_{indirect}$ = 0.1). Spike length had a weak negative indirect effect on grain yield via a reduction of spike
455 fertility ($\sigma_{indirect}$ = -0.1). This spike fertility is also negatively impacted by spike density resulting in a
456 negative indirect effect of spike density on grain yield ($\sigma_{indirect}$ = -0.5). Spike DM at BBCH89 had a
457 weak positive impact on grain yield by a positive contribution to TKW ($\sigma_{indirect}$ = 0.05). Concerning
458 vegetative measurement at beginning of stem elongation (i.e., BBCH30), negative effect was
459 observed on grain yield. Tiller density at BBCH30 had a σ_{direct} of -0.2 and a $\sigma_{indirect}$ via TKW of -0.06 on
460 grain yield, as total DM at BBCH30 had a $\sigma_{indirect}$ of -0.2 via spike fertility and grain density on grain
461 yield. A small negative impact of total DM at BBCH89 was observed on grain yield via a negative
462 influence on TKW ($\sigma_{indirect}$ = -0.06). Finally, plant height had a small positive influence on grain yield
463 with σ_{direct} =0.1 and $\sigma_{indirect}$ = 0.04 via its positive influence on TKW.

464 *Table 4 : (A) Fit indices and (B) variances explained of the path analysis.*

Number of observations	Degrees of freedom	(A) Model fit indices					(B) Variances explained (r^2)				
		SRMR	RMSE	TLI	CFI	X² value	Grain yield	Thousand kernel weight	Grain density	Spike density	Spike fertility
137	7	0.00	0.09	0.96	0.99	0.05	0.87	0.61	0.92	0.98	0.45

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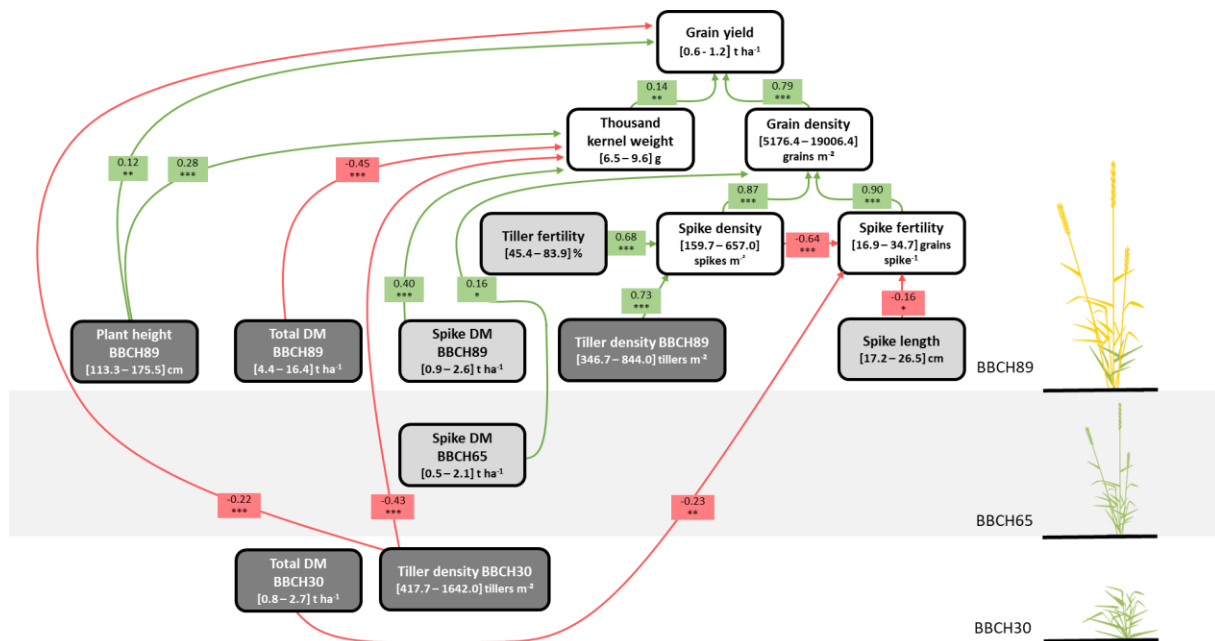
Figure 6: Path coefficients of the final model for the relationship between grain yield and crop measurements, followed by their range values between brackets and units. Groups of traits are colored in different shades of gray, including grain yield and its components (in white), maturity traits (in medium gray) and vegetative traits (in dark gray). A direct path is represented by a single arrow that directly connects two traits (e.g., grain yield and grain density), whereas an indirect path is when the path between a pair of traits is separated by another trait (e.g., grain yield and spike density). Insignificant paths are excluded, statistical significance of the path coefficient at p-value≤0.05 is indicated by ""; at p-value<0.01 by "**" and at p-value<0.001 by "***". Green arrows represent positive contributions and red arrows represent negative contributions.*

4. Discussion

4.1. Plant reproductive allocation and allometry

Resource allocation of *Th. intermedium* towards grains was low; grain yield ranged from 0.6 to 1.4 t ha^{-1} (Figure 6). Although fruiting efficiency ranged from 76 to 136 grains g^{-1}, values of spike DM at flowering and spike harvest index (i.e., ranging from 0.8 to 1.7 t of DM ha^{-1} and from 45 to 75 %, respectively; Table S1), were globally lower compared to mean values of wheat (Hucl & Graf, 1992; Slafer *et al*., 2015; Terrile *et al*., 2017; Rivera-Amado · *et al*., 2019). These weak performances primarily resulted from a low yield per spike (considered as a function of TKW and spike fertility). *Th. intermedium* had a limited capacity to set up large grains; the highest TKW was observed during the establishment year with values not exceeding 9.6 g (Figure 5). Furthermore, the calculated spike fertility was quite weak ranging from 17 to 35 grains spike^{-1} and remained a major contributor to the yield compared to TKW (Figure 6). As observed by Larson *et al*. (2019) floret fertility of *Th. intermedium* didn't exceed 49 %; this was identified as one of the principal limitations for seed production. Altendorf *et al*. (2021) also reported the floret site utilization as the primary contributor to the yield per spike. Floret site utilization is a complex trait that can be influenced by different factors such as pollen availability, self-incompatibility, seed shattering or seed abortion (Altendorf *et al*., 2021). As highlighted by Cattani & Asselin (2018), as a self-incompatible species, selection for uniformity of flowering at optimal time during the day to avoid pollen unavailability and at optimal time during the growing season to avoid post-anthesis heat and drought stress should enhanced the potential for seed set in *Th. intermedium*. Altendorf *et al*. (2021) also suggested possible resource allocation issues. The N harvest index, as an indicator of the N allocated to the grains compared to the total aboveground biomass, ranged from 31 to 58 % (Table S1). In another value range, Mårtensson *et al* (2022) found out that the N harvest index of *Th. intermedium* ranged from 17 % to 24 %. Varying among crop species and genotypes of the same species, the N harvest index in wheat is estimated to be more than 80 % and relatively insensitive to the N fertilizer supply (Hawkesford,

2014). In comparison, the utilization of the N by *Th. intermedium* for grain production is not completely efficient. In our previous study (Fagnant et al., 2023), we showed a lack of accumulation of N and dry matter after anthesis in spikes that can be translated in low grain yields. Previous research on the partitioning of carbohydrate to seed in *Lolium perenne* L. and consequent seed filling suggest that spike has sufficient capacity to fill available seeds without contribution from vegetative tissues (Trethewey & Rolston, 2009). Therefore, more understanding about factors driving the priority of allocation of assimilates and nutrients to each sink during reproductive development of *Th. intermedium* are needed, along with the contribution from each source organs, to enhance its seed sink strength.

From the path analysis (Figure 6), compensation mechanisms were observed between yield per spike and yield per plant as spike fertility was negatively influenced by spike density. Pinto *et al.* (2021) also observed a trade-off with spike weight negatively correlated to spike density of *Th. intermedium*. During the second year, we observed the highest level of tiller density and aboveground biomass at the beginning of the growing season (i.e., BBCH30 stage) compared to the other cropping years (i.e., 1415 tillers m^{-2} and 2.1 t of DM ha^{-1}; Figure 2 and 3). However, such an early biomass and tillering productivity was found detrimental to the yield per spike through spike fertility and TKW (Figure 6) and can further increase the lodging risk (Table S3). The path analysis also revealed that spike length, which was also the highest during the second growing season, had a negative influence on spike fertility. This year, the lowest values of TKW and spike harvest index were observed, and nitrogen harvest index and harvest index dropped to values of 31 % and 6 % (compared to a harvest index of 11 % the other years), respectively (Table S1). This indicated a general relative limited allocation of resources to grain production, and a loss of allocation to seeds on a dry weight basis when the biomass is peaking. As explained by Slafer *et al.* (2022), initial overproductions of organs in wheat - such as tillers - or floret primordia - were followed by their massive mortality. We hypothesize that this negative relationship arises from feedback interactions induced by the competition for a shared limiting growth resource. So, allocating more resources to the survival and growth of many reproductive tillers, hence increasing spike density, implies fewer resources for each individual tiller and spike, and limits their fertility (Yang *et al.*, 2019). In our case, during the second growing season, tillering of *Th. intermedium* was undoubtedly excessive. Many tillers couldn't be supported up to grain harvest and regressed or were weakly productive. However, contrary to Hunter *et al.* (2020a) spike fertility in this study didn't decrease over time that could be explained by our limited spike density (i.e., 660 spikes m^{-2} maximum) compared to Hunter *et al.* (2020a) that observed up to 960 spikes m^{-2}. This spike density was mostly linked to the density of tillers subsisting until grain harvest (Figure 6) and was relatively constant over time (Figure 5). The lower spike density of this study may have limited competition between spikes and maintained relatively higher spike fertility. All in all, this sustains the hypothesis that yield and harvest index decline with stand age such as observed in other studies (Jungers *et al.*, 2017; Pugliese, 2017; Frahm *et al.*, 2018; Tautges *et al.*, 2018; Bajgain *et al.*, 2020; Locatelli *et al.*, 2023) might partly be caused by the increasing competition in stands with excessive tiller density (Law *et al.*, 2020; Cassman & Connor, 2022). While tillering is necessary for aboveground biomass production, selection for grain yield improvement should focus on the reduction of excessive tillering (incl. unfertile tillers) as also highlighted by Altendorf *et al.* (2021) to allow greater resources allocation to the yield per spike through spike fertility and TKW.

4.2. Key drivers of the final grain yield in field

Overall, our study demonstrates for the first time a potential to achieve constant grain yields over four years, with yields at the high end of the range of values known for this crop (Figure 5). The grain density was the major contributor of the final grain yield per hectare (Figure 6), as found with cereal crops (Hay & Porter, 2006). Grain density of *Th. intermedium* was almost evenly associated to spike density and spike fertility (Figure 6). It was positively related to the biomass of spikes reported at the flowering stage (i.e., BBCH65 stage; Figure 6). Actually, the biomass of the spike at anthesis is known

to be a strong proxy of grain density since it indicates a long stem elongation phase, which is often ultimately associated with an increase in seed number. In the absence of stress or other factors affecting grain set, cereal grain yield is source limited with a defined capacity to store assimilate within each grain. The available biomass from post-flowering photosynthesis or from reserves is then allocated to grains up to this limit (Hay & Porter, 2006; Slafer *et al*., 2022).

To maintain grain density and final yield over the years, spike density (reproductive tillers) remained a strong driver since spike fertility ranged similar values from one year to another and TKW had little variability (Figure 5 and 6). However, we observed that when the tiller density is too high and/or resources too scarce, significant mortality and inhibition of reproductive growth of tillers can occur (Figure 3; Figure 5). This was demonstrated in grasses (Meijer & Vreeke, 1988; Svečnjak *et al*., 2022) and has already been observed with *Th. intermedium* (Hunter *et al*., 2020a). Lower grain yields were found linked to a lower number of spikes and the subsequent reduction in the number of grains under the resource-limited conditions. Resource limitations came either from N (non-fertilized plots; Figure 5) or water deficiencies (see Table 2 third year with the lower water reserve during the entire growing season; Figure 5). The importance of reproductive tillers had also been highlighted on the yield per plant in the study of Altendorf *et al*. (2021), where the number of spikes, measured on a per-plant basis, was identified as the primary contributor of yield. Therefore, we hypothesized that in the high-yielding environment of our experiment, i.e., deep and fertile soil conditions coupled with sufficient N fertilization (i.e., 100 kg N ha^{-1} over the growing season) and combined with a good stand establishment (i.e., from 500 to 1000 tillers m^{-2} at the beginning of the growing season; Figure 3) and no overproduction of spikes (i.e., 660 spikes m^{-2} maximum) the crop is able to set up a sufficient number of grains to maintain grain yield potential as stand ages.

4.3. Agronomic levers to enhance grain yield

- #### Nitrogen fertilization

Variable yield responses to N fertilization have previously been reported in the literature, either with increases (Zimbric *et al*., 2021), decreases (Frahm *et al*., 2018) or no impact on *Th. intermedium* grain yield (Dobbratz *et al*., 2023). In our study, the positive grain yield response to N fertilization was found to be mainly related to spike density, tiller fertility and spike DM. Indeed, N fertilization levels around 100 kg N ha^{-1} (either fully applied in early-spring (i.e., BBCH29) or split between autumn and BBCH29) led to spike densities roughly above 400 spikes m^{-2}. However, in the second year the fertilization of 100 kg N ha^{-1} fully applied in early-spring stage was detrimental to grain yield. Meijer & Vreeke (1988) showed that grain yield responses to N fertilization of three perennial grasses (i.e., *Lolium perenne, Poa pratensis* and *Festuca rubra*) was mainly related to the number of inflorescences produced and to a lesser degree to the production of seed per inflorescence. In fact, the timing of N availability must be thought accordingly to the tillering dynamic of the species or genotype grown. Langer (1979) highlighted that seed yields of grasses depend strongly on spike density, and early formed tillers are largely responsible for producing these spikes. For example, with *Dactylis glomerata* L., *Phleum pratense* L. and *Festuca pratensis* Huds. the tillers present in early autumn make up the bulk of the grain yield the following year. However, with *L. perenne* it seems to be possible for a reasonably large proportion of spring-formed tillers to become fertile (Langer, 1979). In addition, early formed inflorescences of *L. perenne* consistently had more spikelets per ear and flowers per spikelet when compared to late-formed ones (Svečnjak *et al*., 2022). So, it is critical to deepen the research on the dynamic of both tillering and N availability to help finding the right conditions for the enhancement of reproductive tiller growth and the mitigation of trade-off with the yield per spike. In this way, study the unique application of N in autumn in comparison to a unique application in spring would be helpful. In our study, N fertilization scheme including 50 kg ha^{-1} applied in autumn combined with 50 kg ha^{-1} applied in early-spring (i.e., BBCH29) (i.e., treatment 3) appears to be a good compromise for optimal grain production by strengthening early tillering in fall,

and sustaining tiller fertility later on, while limiting the risk of excessive biomass production. Still, this rule of thumb should be adapted depending on local conditions and the observed crop development in field. For instance, when *Th. intermedium* shows an important biomass production at the beginning of the season (BBCH30), and the number of tillers is already enough (i.e., tiller density around 1300 tillers m^{-2}), we believe that the early-spring N fraction should be reduced to avoid overproduction of tillers and later mortality or competition effects.

4.3.1. Management of post-harvest crop residues

Few days after each harvest of grains, the straw was exported from the field, and the post-harvest crop residues were shredded at 5 cm from the ground to prevent obstruction of light perception at the bases of *Th. intermedium* plants. This management operation might be at play to explain the relatively constant fertility of the tillers (Table S1). Indeed, previous studies have highlighted a decline in yield with stand age, combined with a decrease in spike density (Jungers *et al*., 2017; Law *et al*., 2020). Authors hypothesized that changes in light quality perceived by the axillary buds located at the bases of the plant, which could reduce reproductive tiller initiation or trigger light avoidance syndrome, might be the cause (Jungers *et al*., 2017; Pinto *et al*., 2021; Rouet *et al*., 2021). Different management practices to increase light penetration to the canopy have previously been tested to maintain *Th. intermedium* grain yields over the long term, such as row spacing (Hunter *et al*., 2020a), between-row plant termination methods (with cultivation or herbicide application; Bergquist, 2019; Law *et al*., 2020; Pinto *et al*., 2021) or within-row suppression methods using burning and mowing (Bergquist, 2019; Hunter *et al*., 2020a; Pinto *et al*., 2021). Inconclusive results were observed: generally, such agronomic practices had the potential to increase yields, but not to prevent yield decline with stand age. In addition, these operations at plot scale could increase tiller and spike fertility, but they were associated with a reduction of other yield components such as spike density, inducing an effective increase of the yield per row without increasing the yield per surface unit (Pinto *et al*., 2021). As observed for other species, the possibility that *Th. intermedium* tillers - present since early autumn - would become the future reproductive tillers having the major impact on grain yield the following year cannot be excluded (Langer, 1979). The optimum time of light perception at tiller bud level for the induction of reproductive tillers and the resulting management practices in link with tiller population regulation remain to be identified with the construction of new knowledge about the tiller fertility dynamic of *Th. intermedium.*

4.3.2. Autumn defoliation

The biomass produced during the autumnal regrowth was the highest during the first year, reaching 1.3 t of DM ha^{-1} for the N treatment with an autumn application (i.e., treatment 3). During the following regrowth years, the levels of biomass produced in autumn were low and could hardly justify a forage harvest. The defoliation performed at autumn tended to decrease the total aboveground biomass observed the subsequent growing season. While reducing tiller density at grain harvest, the autumn defoliation had no impact on grain yield as compensation, with higher TKW, was reported. These results are in line with previous studies where forage harvest, when performed in summer (i.e., after grain harvest) or in autumn (Zimbric *et al*., 2021) didn't impact grain yield or even increased it (Pugliese *et al*., 2019; Culman *et al*., 2023). In addition, the study of Dick *et al*. (2018) highlighted that the grazing of residues increased seed yield and harvest index compared to the removal or chopping of residues. However, Zimbric *et al*. (2021) highlighted that when a spring forage harvest was performed, it decreased grain yield. Pugliese *et al*. (2019) observed that even if the grain yield was increased with summer and/or autumn forage harvest, it didn't prevent the yield decline with stand age. Therefore, when aboveground biomass appears sufficient after grain harvest, we are convinced that a defoliation performed at autumn could be performed without hampering grain production and may help to maintain the yield per spike.

5. Conclusions

Th. intermedium is characterized by a limited allocation to grains compared to marketed annual cereals. However, we demonstrated for the first time that grain yield potential can be maintained as crop ages thanks to constant tiller fertility and spike fertility. Fertile environment favored by N fertilization combined with optimal stand establishment, early induced tillers and no overproduction of spikes were found crucial in this regard. Due to the potential mortality and inhibition of reproductive growth of tillers combined with the negative influence of early tiller density and aboveground biomass on the yield per spike, excessive aboveground production at the beginning of the reproductive phase should be avoided. In this way, N fertilization of 50 kg ha^{-1} applied in autumn combined with a fertilization of 50 kg ha^{-1} applied in early-spring supported tiller fertility over time while limiting the risk of excessive biomass production. Whereas autumn plant regrowth was only sufficient to perform a forage harvest the first year of growth, the autumn defoliation didn't reduce grain yield and allowed an additional valorization of the aboveground biomass. Finally, shredding post-harvest crop residues at ground level to prevent obstruction of light perception at the bases of plants may have a positive influence on the induction of reproductive tillers. In the future, greater resources allocation to the grains should be achieved by breeding through the increase of the yield per spike and the limitation of tiller production, whether by tillering or rhizome propagation, without compromising the spike density, the vigor of regrowth and its environmental performances.

CRediT authorship contribution statement

L.F., B.D.: Conceptualization and planning of the experiments. Formulation of research goals and aims.

L.F.: carrying out the samplings, data curation, formal analyses (statistical and mathematical).

B.D.: Supervision.

B.D., L.F.: Development and design of methodology.

L.F., B.D., F.C., O.D., contributed to the interpretation of result.

L.F.: Writing – original draft Preparation.

F.C., O.D., B.D.: help provided for data presentation and visualization, critical review, commentary and revision, validation.

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Supplementary material

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900 *Table S1: Marginal means ± standards errors of some crop measurements from 2018 to 2021 on the four common autumn non-defoliated N treatments. "/" is indicating that the variable was not measured on this treatment. Letters represent the results of the post hoc analysis of the effect of (A) N fertilization or (B) stand age.*

	(A) N fertilization				(B) Stand age			
	0+0+0N	50+0+0N	50+0+50N	100+0+0N	2018	2019	2020	2021
Plant height (cm)	146±2b	152±2ab	156±2a	158±2a	149±2c	173±2a	125±2d	164±2b
Spike length (cm)	19±1	21±1	22±1	22±2	22±1ab	24±1a	19±1b	20±1b
Spike DM BBCH65 (t DM ha^{-1})	0.8±0.1c	1.0±0.1bc	1.3±0.1a	1.2±0.1ab	0.8±0.1c	1.7±0.1a	0.8±0.1c	1.1±0.1b
Spike DM BBCH89 (t DM ha^{-1})	1.3±0.1b	1.6±0.1ab	1.5±0.1ab	1.9±0.1a	1.5±0.2b	2.2±0.1a	1.1±0.1b	1.5±0.1b
Tiller fertility (%)	59±4b	73±4a	73±4a	72±4a	70±4a	77±4a	56±4b	74±4a
Harvest index (%)	11±1a	10±1ab	11±1ab	8±1b	12±1a	6±1b	11±1a	10±1a
Nitrogen harvest index (%)	51±5	50±6	46±6	40±6	/	31±5b	51±5a	58±5a
Fruiting efficiency (grains g^{-1})	135±11a	127±11ab	102±11b	96±11b	98±11b	76±11b	150±10a	136±10a
Spike harvest index (%)	75±9a	67±9ab	65±9ab	54±9b	67±9a	45±9b	74±8a	75±9a

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Table S2: Correlation coefficients and their significance between vegetative components.

		Total DM						Spike DM		Tiller density		Plant Height
		Previous autumn	BBCH30	BBCH39	BBCH65	BBCH89	Next autumn	BBCH65	BBCH89	BBCH30	BBCH89	
Total DM	Previous autumn	1	0.59	0.75	0.76	0.74	/	0.57	0.37	0.68	0.21	0.62
			***	***	***	***		***	**	***		***
	BBCH30		1	0.45	0.40	0.35	-0.2	0.33	0.19	0.36	0.29	0.11
				***	***	***		***	*	***	***	
	BBCH39			1	0.73	0.65	-0.04	0.64	0.45	0.66	0.27	0.46
					***	***		***	***	***	***	***
	BBCH65				1	0.79	-0.43	0.93	0.57	0.69	0.39	0.65
						***	***	***	***	***	***	***
	BBCH89					1	-0.23	0.75	0.81	0.59	0.53	0.68
								***	***	***	***	***
	Next autumn						1	-0.24	0.01	-0.35	-0.66	-0.2
								*		**	***	
Spike DM	BBCH65							1	0.64	0.58	0.30	0.64
									***	***	**	***
	BBCH89								1	0.36	0.42	0.51
										**	***	***
Tiller density	BBCH30									1	0.37	0.37
											***	***
	BBCH89										1	0.23
												**
Plant Height												1

904   *Table S3 : Significant correlation coefficients and their significance between crop measurements and lodging.*

	Grain yield	Spike fertility	Harvest index	N harvest index	Fruiting efficiency	Spike DM BBCH65	Tiller density BBCH30	Plant Height	Total DM					
									Previous autumn	BBCH30	BBCH39	BBCH65	BBCH89	Next autumn
Lodging	-0.24	-0.22	-0.33	-0.36	-0.54	0.4	0.39	0.23	0.35	0.45	0.56	0.52	0.28	0.56
	*	*	**	*	***	***	***	*	*	***	***	***	**	*

Note. "*" indicating statistical significance at *p-value*≤0.05; "**" indicating statistical significance at *p-value*≤0.01; "***" indicating statistical significance at *p-value*≤0.001.

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Table S4: Significant direct and indirect path coefficients and their significance for crop measurements as they contribute to grain yield.

	Path coefficient	p-value
TKW vs grain yield		
Direct effect	0.14	**
Grain density vs grain yield		
Direct effect	0.79	***
Spike fertility vs grain yield		
Indirect effect via *grain density*	0.71	***
Tiller density at BBCH30 vs grain yield		
Direct effect	-0.22	***
Indirect effect via TKW	-0.06	**
Total DM at BBCH30 vs grain yield		
Indirect effect via *spike fertility* and *grain density*	-0.17	**
Tiller density at BBCH89 vs grain yield		
Indirect effect via *spike density* and *grain density*	0.50	***
Total DM at BBCH89 vs grain yield		
Indirect effect via TKW	-0.06	*
Plant height vs grain yield		
Direct effect	0.12	**
Indirect effect via TKW	0.04	*
Spike DM at BBCH65 vs grain yield		
Indirect effect via *grain density*	0.13	*
Spike DM at BBCH89 vs grain yield		
Indirect effect via TKW	0.05	**
Tiller fertility vs grain yield		
Indirect effect via *spike density* and *grain density*	0.47	***
Spike density vs grain yield		
Indirect effect via *grain density*	0.69	***
Indirect effect via *spike fertility* and *grain density*	-0.46	***

| *Spike length vs grain yield* | | |
| Indirect effect via *spike fertility* | -0.11 | 0.05 |

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