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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 al.*,
80 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,

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

109 Beside breeding, Bajgain *et al.* (2022) estimated that 31 % of global yield increase would be linked to
110 better management in fields, as it was seen for wheat. Among this, nitrogen (N) management is one
111 major factor. N availability influences crop yield mostly through its effect upon canopy expansion,
112 survival, and longevity of organs (Satorre & Slafer, 1999; Hay & Porter, 2006). The effect on tiller
113 development, survival and growth in perennial grasses has long been demonstrated (Gislum &
114 Griffith, 2005; Assuero & Tognetti, 2010). When seeking to maximizing grain production, N shortage
115 in autumn or early-spring can be detrimental to yields due to source-limited conditions for tiller
116 development, growth, and spikelet's formation (Langer, 1979; Boelt & Studer, 2010; Reynolds *et al.*,
117 2022). However, the effect of N fertilization for seed-bearing grasses is controversial due to unclear
118 effect on tiller and spike fertility, increased lodging risk or secondary tillering (Langer, 1979; Boelt &
119 Studer, 2010). Some studies have investigated the impact of N fertilization on *Th. intermedium*
120 performance (Jungers *et al.*, 2017; Frahm *et al.*, 2018; Fagnant *et al.*, 2023) and suggested an optimal
121 N fertilization ranging from 61 to 100 kg N ha⁻¹. Defoliation operations can have a major impact on
122 grass productivity as well. Defoliation performed above the apex height is known to preserve the
123 tiller reproductive potential. Meanwhile, defoliation may generate new vegetative tillers and could
124 eventually delay stem elongation or reduce tiller height (Mansat & Pfitzenmeyer, 1966; Gillet, 1973;
125 Rouet *et al.*, 2021). With respect to *Th. intermedium*, various management options have been
126 studied to improve grain yield in young stands or to maintain yield in aging stands including crop
127 defoliation in summer (i.e., just after grain harvest), in autumn or in spring, either by grazing (Dick *et al.*,
128 2018) or mowing. As the spring mowing tends to decrease grain yield (Zimbric *et al.*, 2021;
129 Culman *et al.*, 2023), the summer and autumn mowing can increase grain yield compared to no
130 forage harvesting (Pugliese *et al.*, 2019; Culman *et al.*, 2023), but without mitigating yield decline
131 with stand age (Hunter *et al.*, 2020a).

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

138 2. Material and methods

139 2.1. Experimental design

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

144 treatments: i) no autumn defoliation and ii) autumn defoliation. During autumn mechanical
 145 defoliation vegetation was clipped to a height of 7 cm and removed simultaneously. Defoliation was
 146 only performed in the autumn (i.e., early November) of the first and third cropping year due to
 147 insufficient plant regrowth the other years (Table 1). The N fertilization treatments (ammonium
 148 nitrate granular) differed according to the total amount and the timing of application and were
 149 modified during the study with only some N treatments present during the four cropping years
 150 (Table 1). Each subplot (i.e., split plot of 4 * 8 m) was divided by two to obtain one plot being
 151 dedicated to grain yield measurement in summer and the other one to destructive sampling during
 152 the growing season. The experiment was sown on September 22nd, 2017, with an interrow spacing of
 153 25cm and a density of 250 seeds m⁻² leading to a plant density of 176 plants m⁻² in the first cropping
 154 year. Seeds used originate from the fifth breeding cycle achieved at The Land Institute (Kansas, USA).
 155 The field is characterized by deep and fertile soil conditions with a clay-loam soil type, and optimal
 156 nutrients content with a phosphorus content averaging 0.16 mg g⁻¹, potassium content averaging
 157 0.22 mg g⁻¹ and organic carbon content averaging 13 g kg⁻¹ over the four years of the experiment.
 158 Chemical weeding (i.e., 80 g ha⁻¹ of clopyralid, 120 g ha⁻¹ of fluoxypyr and 800 g ha⁻¹ of MCPA on
 159 November 17th, 2017, coupled with 960 l ha⁻¹ of bentazon and 30 g ha⁻¹ of amidosulfuron on April
 160 18th, 2018) and two mechanical weeding (i.e., interrow hoe weeder in late May 2018) were only
 161 performed during the first cropping year. Most post-harvest residues (i.e., straw) were removed
 162 manually from the field and the remaining part was shredded at 5 cm from the ground after each
 163 grain harvest.

164 *Table 1 : (A) N fertilization treatments described with their timings and amounts (kg ha⁻¹) and (B) defoliation treatments of*
 165 *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					2018	2019	2020	2021	
Treatment number	Total N dose (kg N ha ⁻¹)	Splitting (kg N ha ⁻¹)							
		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			Path analysis
6	150	100	50	0			x	x	
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.

166 2.2. Data collection

167 Data were collected during the four cropping years of the experiment (representing four grain
 168 production years with a first establishment year and three successive regrowing years). Aboveground
 169 biomass was sampled with a 50 x 50 cm quadrat per plot, cut at 5 cm above soil surface, oven-dried
 170 (72 h at 60 °C) and weighted to obtain dry matter (DM). Samples were collected at five different main
 171 phenological stages during the growing season; four reproductive stages rated with the BBCH scale
 172 (Meier, 2018), namely the stem elongation (BBCH30), the flag leaf (BBCH39), the flowering (BBCH65)
 173 and the grain maturity (BBCH89) stages as well as a vegetative stage during the autumn regrowth.
 174 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
$$\text{spike fertility} = \frac{\text{grain yield}}{\text{spike density} \cdot \frac{\text{TKW}_{un}}{1000}} \text{ (Equation 1)}$$

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

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.

203 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

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

252 2.4.2. Correlation analysis

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

261 2.4.3 Path analysis

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

266 2.4.3.1. Development of the initial model

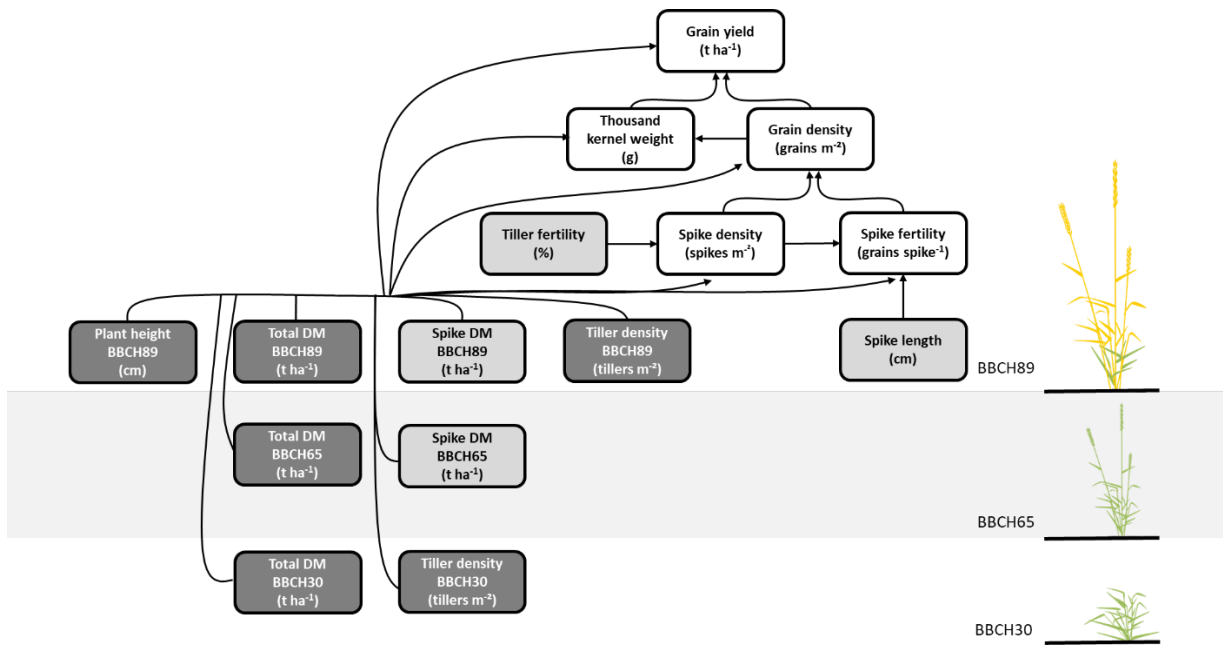
267 The initial model described in Figure 1 was designed with the assumption and understanding that
268 yield components develop sequentially, and that their effects on one another are unidirectional (i.e.,
269 represented by the sense of the arrow; Figure 1), meaning that later developing components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

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

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

288 2.4.3.2. Model fitting

289 To assess that the model fits the data, Chi-square test (χ^2) was used, where an insignificant p-value
 290 (p -value>0.05) indicates an acceptable model. Multiple alternative indices were also used; the
 291 comparative fit index (CFI) with value above 0.90, the Tucker-Lewis index (TLI) with value above 0.95,
 292 the root means square error approximation (RMSEA) as well as the standardized root mean square
 293 residual (SRMR) with value below 0.08 are generally indicating a good model fit.



294
 295 *Figure 1 : Initial structural equation model for the relationship between grain yield and crop measurements, followed by their units*
 296 *in parentheses. Groups of traits are colored in different shades of gray, including grain yield and its components (in white), maturity*
 297 *traits (in medium gray) and vegetative traits (in dark gray). A direct path is represented by a single arrow that directly connects two*
 298 *traits (e.g., grain yield and grain density), whereas an indirect path is when the path between a pair of traits is separated by*
 299 *another trait (e.g., grain yield and spike density).*

300 3. Results

301
302

Table 3 : Significance levels from the two-way ANOVA on the four common N treatments for the various crop measurements. (A) the first ANOVA performed on the year 2019 and 2020 considered as random effect and (B) the second ANOVA performed on the autumn non-defoliated treatment and the four cropping years considered as fixed effect through the stand age.

Variables	(A) Two-way ANOVA			(B) Two-way ANOVA		
	Defoliation (D)	N fertilization (N)	D x N	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 $p\text{-value}\leq 0.05$; “***” indicating statistical significance at $p\text{-value}\leq 0.01$; “****” indicating statistical significance at $p\text{-value}\leq 0.001$. “/” indicating that the variable was not tested in the ANOVA.

303

304

305 3.1. Aboveground development

306 3.1.1. Impact of stand age and phenological stage

307 As shown in Figure 2, total aboveground biomass varied considerably across stand age (Table 3B). We
308 observed the highest level of total DM during the second reproductive phase (i.e., 2019), reaching a
309 mean of 15.8 ± 0.6 t of DM ha⁻¹ at grain maturity stage (i.e., BBCH89). The three other reproductive
310 phases (i.e., 2018, 2020 and 2021) presented similar levels of total DM at BBCH89 stage ranging from
311 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
312 treatment (i.e., treatment 4 that is sufficient to achieve a N non-limited crop development see
313 Fagnant *et al.*, (2023)). Total DM produced during the autumn regrowth was low and also
314 significantly influenced by the stand age (Table 3B), with the highest level observed after the first
315 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
316 for the N-control treatment; Figure 2).

317 Tiller density during the different reproductive phases is presented in Figure 3. Tiller density at the
318 beginning of stem elongation (i.e., BBCH30) varied significantly across stand age (Table 3B), with the
319 lowest level observed during the first year (i.e., 2018, with 463 ± 52 tillers m⁻² on average) and the
320 highest during the second year (i.e., 2019, with 1415 ± 48 tillers m⁻² on average). By contrast, tiller
321 density at BBCH89 stage was globally not influenced by the stand age (Table 3B). An exception can be
322 mentioned with the N-control treatment where the highest tiller density at BBCH89 stage was
323 observed in 2019 (p-value<0.001) due to particularly low tiller mortality (i.e., the decrease of tiller
324 density during the reproductive phase; Figure 3). Tiller mortality was the highest during the second
325 year and the lowest during the first year. As opposed to the other years, tiller mortality was not
326 observed in the first year as tiller density increased from BBCH30 to BBCH89 stages (Figure 3; Table
327 3B).

328 Plant height was significantly impacted by stand age (Table 3B). The tallest plants were observed in
329 the second year (i.e., 2019 with a mean of 173 ± 2 cm) and the lowest were observed in 2020 (i.e.,
330 125 ± 2 cm on average), marginal means are presented in supplementary material (Table S1).

331 3.1.2. Impact of N fertilization

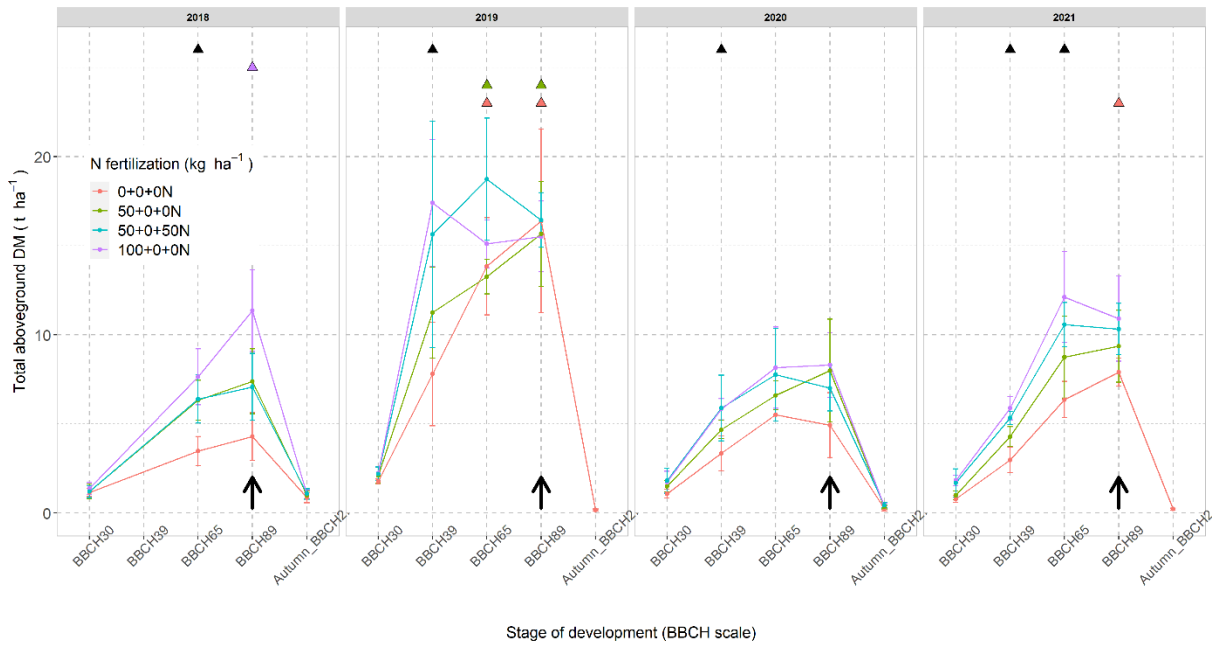
332 Total aboveground biomass varied considerably across N fertilizations (Table 3B). Globally, the N-
333 control treatment had always the lowest total DM and moderate early-spring fertilization (i.e.,
334 treatment 2) appeared inadequate to increase total DM during the reproductive phase contrary to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

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

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

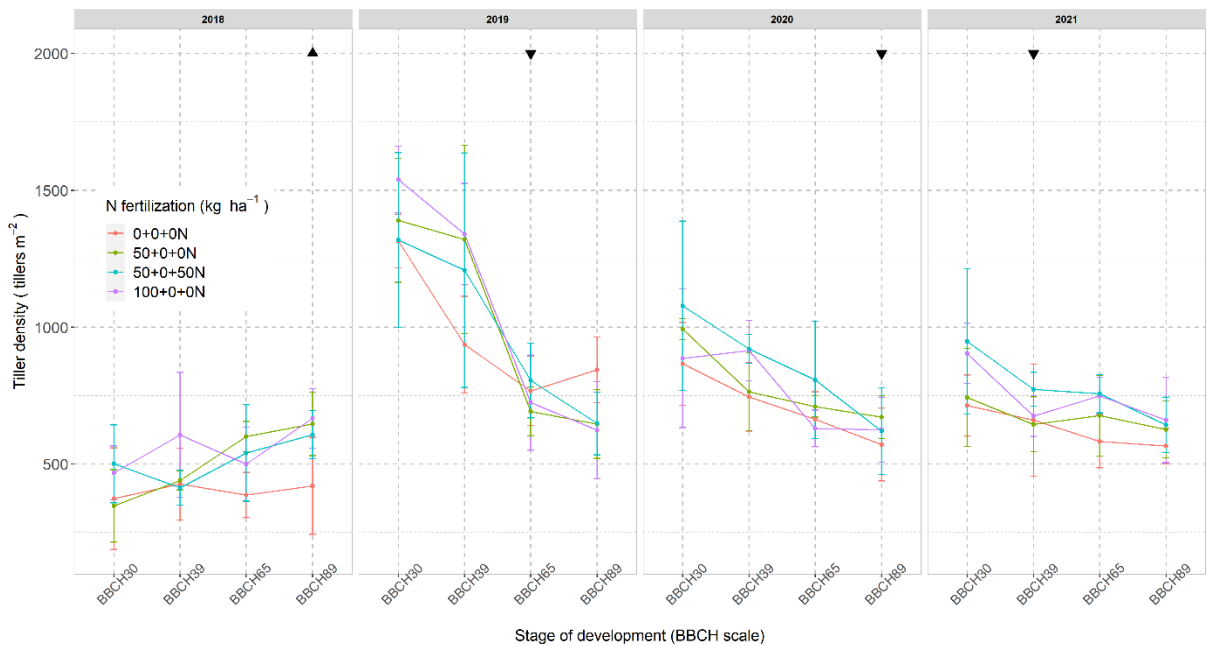
343 3.1.3. Impact of defoliation

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



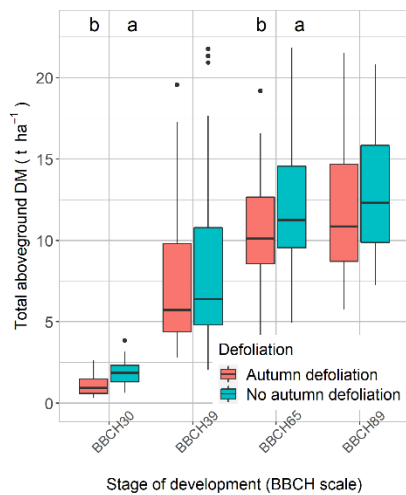
349

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



355

356 *Figure 3: Mean dynamic of tiller density during the reproductive phase from 2018 to 2021 on the autumn non-defoliated*
 357 *treatment. Standard errors are represented by error bars. Statistical differences (p -value<0.05; post hoc analysis) between*
 358 *phenological stages are indicated by Δ specifying an increased value; and ∇ specifying a decreased value compared to the*
 359 *previous phenological stage. The color of the symbols represents the N treatment concerned and symbols in black represents*
 360 *the same trends for all N treatments.*



361

362 *Figure 4 : Effect of autumn defoliation on the total aboveground biomass during the reproductive phase of 2019 and 2021.*
 363 *Letters represent the results of the post hoc analysis (p-value<0.05) of the effect of autumn defoliation on each phenological*
 364 *stage.*

365 3.1.4. Relationship between aboveground development traits

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

374 3.2. Grain production

375 3.2.1. Impact of stand age

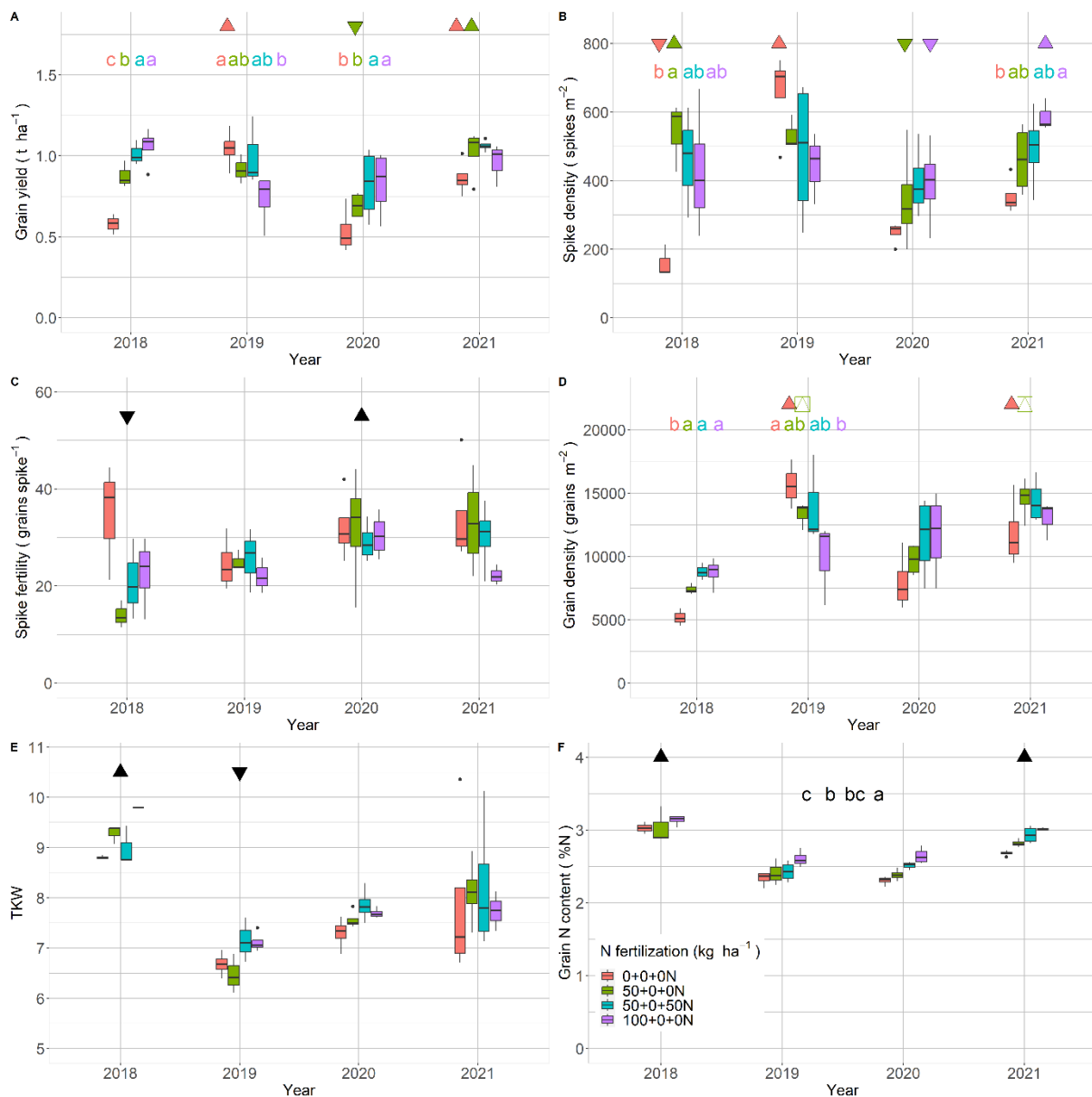
376 Grain yield, spike density, grain density, spike fertility, thousand kernel weight (TKW) and grain N
 377 content through the different stand ages are presented in Figure 5 and marginal means of spike DM,
 378 tiller fertility and spike length are provided in supplementary material (Table S1). According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

394 3.2.2. Impact of N fertilization

395 The effect of N fertilization was dependent on the stand age for grain yield, spike density and grain
396 density (Table 3B). Concerning grain yield and grain density this effect was significant in 2018, 2019
397 and 2020 with contrasting results. The effect of N fertilization was positive in 2018 and 2020 and
398 negative in 2019. Contrary to 2018 and 2020, a higher grain yield and grain density was observed in
399 2019 with the N-control treatment (p-value<0.05; Figure 5). Spike density was positively influenced
400 by N fertilization in 2018 and 2021 (p-value<0.01; Figure 5). Globally tiller fertility, spike length, spike
401 biomass and N content in grains had reduced level with the N-control treatment contrary to the N-
402 sufficient treatments (i.e., treatment 3 and 4) that increased their levels (Table 3B; Table S1; Figure
403 5). Spike fertility and TKW were not influenced by N fertilization (Table 3B)

404 3.2.3. Impact of defoliation

405 Defoliation was only compared in 2019 and 2021, the N content in grains was not measured between
406 defoliation treatments. TKW was the only grain yield components influenced by defoliation in
407 autumn with an increase of 1 g when an autumn defoliation was performed (Table 3A).



408

409 *Figure 5 : Effect of N fertilization and stand age on grain yield, its components and grain N content on the autumn non-*
 410 *defoliated treatment from 2018 to 2021. Letters represents the results of the post hoc analysis (p-value<0.05) of the effect*
 411 *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*
 412 *specifying an increased value; ◻ specifying an equal increased value; ▽ specifying a decreased value compared to the other*
 413 *cropping years. The color of the symbols and letters represents the N treatment concerned when there is an interaction*
 414 *between stand age and N fertilization, otherwise the symbols and letters are in black.*

415 3.2.4. Partitioning to reproductive organs

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

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

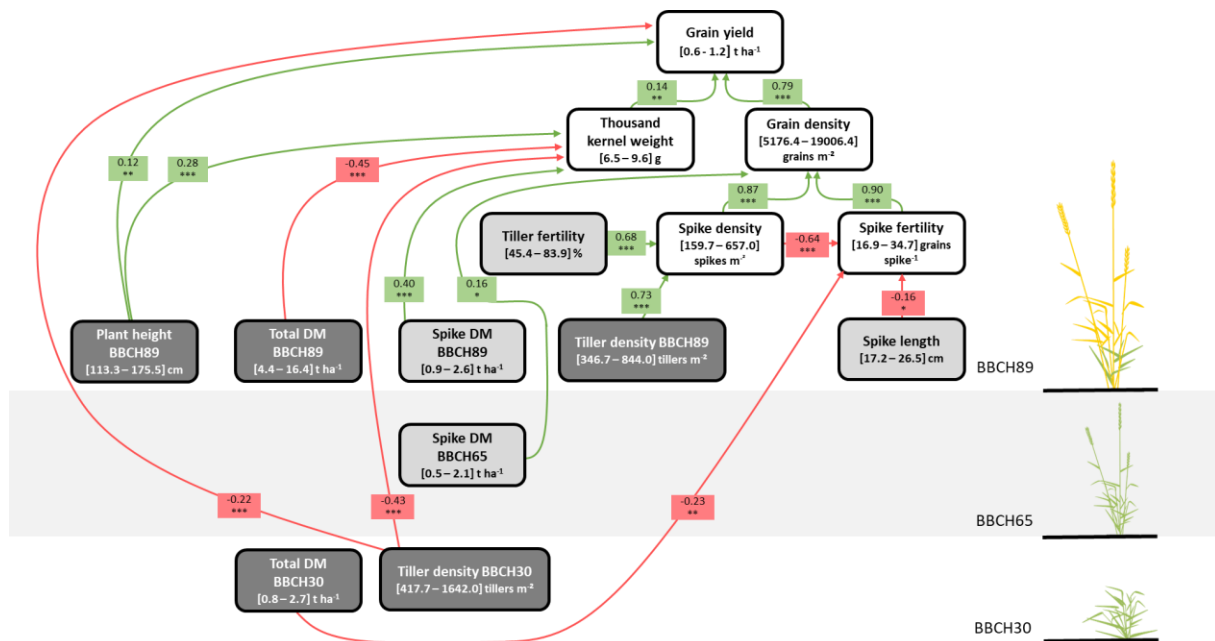
442 Acceptable fit indices (see section 'Model fitting' of the material and methods) were observed for
 443 path analysis that confirms that the specified model fitted well the data. Fit indices and the variance
 444 explained are shown in Table 4. The path coefficients and their significance are shown 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 (σ_{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 $\sigma_{\text{direct}} = 0.8$ 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 ($\sigma_{\text{indirect}} = 0.7$). 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 ($\sigma_{\text{indirect}} = 0.5$). Spike DM at
 453 flowering (i.e., BBCH65) had a small indirect effect on grain yield by an increase of grain density
 454 ($\sigma_{\text{indirect}} = 0.1$). Spike length had a weak negative indirect effect on grain yield via a reduction of spike
 455 fertility ($\sigma_{\text{indirect}} = -0.1$). This spike fertility is also negatively impacted by spike density resulting in a
 456 negative indirect effect of spike density on grain yield ($\sigma_{\text{indirect}} = -0.5$). Spike DM at BBCH89 had a
 457 weak positive impact on grain yield by a positive contribution to TKW ($\sigma_{\text{indirect}} = 0.05$). 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 σ_{indirect} via TKW of -0.06 on
 460 grain yield, as total DM at BBCH30 had a σ_{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 ($\sigma_{\text{indirect}} = -0.06$). Finally, plant height had a small positive influence on grain yield
 463 with $\sigma_{\text{direct}} = 0.1$ and $\sigma_{\text{indirect}} = 0.04$ 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	χ^2 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

465



466

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

474 4. Discussion

475 4.1. Plant reproductive allocation and allometry

476 Resource allocation of *Th. intermedium* towards grains was low; grain yield ranged from 0.6 to 1.4 t
 477 ha⁻¹ (Figure 6). Although fruiting efficiency ranged from 76 to 136 grains g⁻¹, values of spike DM at
 478 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;
 479 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
 480 primarily resulted from a low yield per spike (considered as a function of TKW and spike fertility). *Th.*
 481 *intermedium* had a limited capacity to set up large grains; the highest TKW was observed during the
 482 establishment year with values not exceeding 9.6 g (Figure 5). Furthermore, the calculated spike
 483 fertility was quite weak ranging from 17 to 35 grains spike⁻¹ and remained a major contributor to the
 484 yield compared to TKW (Figure 6). As observed by Larson *et al.* (2019) floret fertility of *Th.*
 485 *intermedium* didn't exceed 49 %; this was identified as one of the principal limitations for seed
 486 production. Altendorf *et al.* (2021) also reported the floret site utilization as the primary contributor
 487 to the yield per spike. Floret site utilization is a complex trait that can be influenced by different
 488 factors such as pollen availability, self-incompatibility, seed shattering or seed abortion (Altendorf *et al.*,
 489 2021). As highlighted by Cattani & Asselin (2018), as a self-incompatible species, selection for
 490 uniformity of flowering at optimal time during the day to avoid pollen unavailability and at optimal
 491 time during the growing season to avoid post-anthesis heat and drought stress should enhanced the
 492 potential for seed set in *Th. intermedium*. Altendorf *et al.* (2021) also suggested possible resource
 493 allocation issues. The N harvest index, as an indicator of the N allocated to the grains compared to
 494 the total aboveground biomass, ranged from 31 to 58 % (Table S1). In another value range,
 495 Mårtensson *et al.* (2022) found out that the N harvest index of *Th. intermedium* ranged from 17 % to
 496 24 %. Varying among crop species and genotypes of the same species, the N harvest index in wheat is
 497 estimated to be more than 80 % and relatively insensitive to the N fertilizer supply (Hawkesford,
 498

499 2014). In comparison, the utilization of the N by *Th. intermedium* for grain production is not
500 completely efficient. In our previous study (Fagnant et al., 2023), we showed a lack of accumulation
501 of N and dry matter after anthesis in spikes that can be translated in low grain yields. Previous
502 research on the partitioning of carbohydrate to seed in *Lolium perenne* L. and consequent seed filling
503 suggest that spike has sufficient capacity to fill available seeds without contribution from vegetative
504 tissues (Trethewey & Rolston, 2009). Therefore, more understanding about factors driving the
505 priority of allocation of assimilates and nutrients to each sink during reproductive development of
506 *Th. intermedium* are needed, along with the contribution from each source organs, to enhance its
507 seed sink strength.

508 From the path analysis (Figure 6), compensation mechanisms were observed between yield per spike
509 and yield per plant as spike fertility was negatively influenced by spike density. Pinto *et al.* (2021)
510 also observed a trade-off with spike weight negatively correlated to spike density of *Th. intermedium*.
511 During the second year, we observed the highest level of tiller density and aboveground biomass at
512 the beginning of the growing season (i.e., BBCH30 stage) compared to the other cropping years (i.e.,
513 1415 tillers m⁻² and 2.1 t of DM ha⁻¹; Figure 2 and 3). However, such an early biomass and tillering
514 productivity was found detrimental to the yield per spike through spike fertility and TKW (Figure 6)
515 and can further increase the lodging risk (Table S3). The path analysis also revealed that spike length,
516 which was also the highest during the second growing season, had a negative influence on spike
517 fertility. This year, the lowest values of TKW and spike harvest index were observed, and nitrogen
518 harvest index and harvest index dropped to values of 31 % and 6 % (compared to a harvest index of
519 11 % the other years), respectively (Table S1). This indicated a general relative limited allocation of
520 resources to grain production, and a loss of allocation to seeds on a dry weight basis when the
521 biomass is peaking. As explained by Slafer *et al.* (2022), initial overproductions of organs in wheat -
522 such as tillers - or floret primordia - were followed by their massive mortality. We hypothesize that
523 this negative relationship arises from feedback interactions induced by the competition for a shared
524 limiting growth resource. So, allocating more resources to the survival and growth of many
525 reproductive tillers, hence increasing spike density, implies fewer resources for each individual tiller
526 and spike, and limits their fertility (Yang *et al.*, 2019). In our case, during the second growing season,
527 tillering of *Th. intermedium* was undoubtedly excessive. Many tillers couldn't be supported up to
528 grain harvest and regressed or were weakly productive. However, contrary to Hunter *et al.* (2020a)
529 spike fertility in this study didn't decrease over time that could be explained by our limited spike
530 density (i.e., 660 spikes m⁻² maximum) compared to Hunter *et al.* (2020a) that observed up to 960
531 spikes m⁻². This spike density was mostly linked to the density of tillers subsisting until grain harvest
532 (Figure 6) and was relatively constant over time (Figure 5). The lower spike density of this study may
533 have limited competition between spikes and maintained relatively higher spike fertility. All in all,
534 this sustains the hypothesis that yield and harvest index decline with stand age such as observed in
535 other studies (Jungers *et al.*, 2017; Pugliese, 2017; Frahm *et al.*, 2018; Tautges *et al.*, 2018; Bajgain *et al.*,
536 2020; Locatelli *et al.*, 2023) might partly be caused by the increasing competition in stands with
537 excessive tiller density (Law *et al.*, 2020; Cassman & Connor, 2022). While tillering is necessary for
538 aboveground biomass production, selection for grain yield improvement should focus on the
539 reduction of excessive tillering (incl. unfertile tillers) as also highlighted by Altendorf *et al.* (2021) to
540 allow greater resources allocation to the yield per spike through spike fertility and TKW.

541 4.2. Key drivers of the final grain yield in field

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

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

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

570 4.3. Agronomic levers to enhance grain yield

571 • Nitrogen fertilization

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

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

602 4.3.1. Management of post-harvest crop residues

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

626 4.3.2. Autumn defoliation

627 The biomass produced during the autumnal regrowth was the highest during the first year, reaching
628 1.3 t of DM ha⁻¹ for the N treatment with an autumn application (i.e., treatment 3). During the
629 following regrowth years, the levels of biomass produced in autumn were low and could hardly
630 justify a forage harvest. The defoliation performed at autumn tended to decrease the total
631 aboveground biomass observed the subsequent growing season. While reducing tiller density at
632 grain harvest, the autumn defoliation had no impact on grain yield as compensation, with higher
633 TKW, was reported. These results are in line with previous studies where forage harvest, when
634 performed in summer (i.e., after grain harvest) or in autumn (Zimbric *et al.*, 2021) didn't impact grain
635 yield or even increased it (Pugliese *et al.*, 2019; Culman *et al.*, 2023). In addition, the study of Dick *et al.*
636 (2018) highlighted that the grazing of residues increased seed yield and harvest index compared
637 to the removal or chopping of residues. However, Zimbric *et al.* (2021) highlighted that when a spring
638 forage harvest was performed, it decreased grain yield. Pugliese *et al.* (2019) observed that even if
639 the grain yield was increased with summer and/or autumn forage harvest, it didn't prevent the yield
640 decline with stand age. Therefore, when aboveground biomass appears sufficient after grain harvest,
641 we are convinced that a defoliation performed at autumn could be performed without hampering
642 grain production and may help to maintain the yield per spike.

643 5. Conclusions

644 *Th. intermedium* is characterized by a limited allocation to grains compared to marketed annual
645 cereals. However, we demonstrated for the first time that grain yield potential can be maintained as
646 crop ages thanks to constant tiller fertility and spike fertility. Fertile environment favored by N
647 fertilization combined with optimal stand establishment, early induced tillers and no overproduction
648 of spikes were found crucial in this regard. Due to the potential mortality and inhibition of
649 reproductive growth of tillers combined with the negative influence of early tiller density and
650 aboveground biomass on the yield per spike, excessive aboveground production at the beginning of
651 the reproductive phase should be avoided. In this way, N fertilization of 50 kg ha⁻¹ applied in autumn
652 combined with a fertilization of 50 kg ha⁻¹ applied in early-spring supported tiller fertility over time
653 while limiting the risk of excessive biomass production. Whereas autumn plant regrowth was only
654 sufficient to perform a forage harvest the first year of growth, the autumn defoliation didn't reduce
655 grain yield and allowed an additional valorization of the aboveground biomass. Finally, shredding
656 post-harvest crop residues at ground level to prevent obstruction of light perception at the bases of
657 plants may have a positive influence on the induction of reproductive tillers. In the future, greater
658 resources allocation to the grains should be achieved by breeding through the increase of the yield
659 per spike and the limitation of tiller production, whether by tillering or rhizome propagation, without
660 compromising the spike density, the vigor of regrowth and its environmental performances.
661

662 CRediT authorship contribution statement

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

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

666 B.D.: Supervision.

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

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

669 L.F.: Writing – original draft Preparation.

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

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898 Supplementary material

899 *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*
 900 *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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903 *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	Plant Height
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

Note. “*” indicating statistical significance at $p\text{-value}\leq 0.05$; “**” indicating statistical significance at $p\text{-value}\leq 0.01$; “***” indicating statistical significance at $p\text{-value}\leq 0.001$.

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	Tiller density	Plant Height	Total DM					
						BBCH65	BBCH30		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 $p\text{-value}\leq 0.05$; “**” indicating statistical significance at $p\text{-value}\leq 0.01$; “***” indicating statistical significance at $p\text{-value}\leq 0.001$.

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

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

Spike length vs grain yield

Indirect effect via *spike fertility*

-0.11

0.05

Note. “*” indicating statistical significance at $p\text{-value}\leq 0.05$; “**” indicating statistical significance at $p\text{-value}\leq 0.01$; “***” indicating statistical significance at $p\text{-value}\leq 0.001$.

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