Seasonal allocation of dry matter and nitrogen in *Th. intermedium* across stand ages.

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Abstract

Thinopyrum intermedium is currently proposed as a perennial grain for both forage and grain production. Undergoing domestication, its grain yields are low, while its long-lasting organs are ensuring environmental benefits. However, understanding the resource allocation dynamics of *Th. intermedium* is needed. Dry matter (DM) and nitrogen (N) allocations within the different plant parts were quantified over the growing season on various experimental sites and stand ages. Low resource mobilization to spikes was observed after flowering, contrarily to N allocation within stem bases. Indeed, root production and stem bases thickening over the years represented significant N sinks. In addition, the total N within the plant, weakly allocated to spikes (i.e., 10 to 26 %), can decrease at the end of the growing season (i.e., from 34 to 56 kg ha^{-1}). This could be explained by root turnover and release of N-rich root exudates to the soil. With a low exportation of N at grain maturity, averaging 60 kg ha^{-1}, a strategy of nutrient conservation was highlighted. Furthermore, through a small proportion of rhizomes, *Th. intermedium* is characterized by a conservative phalanx growth strategy. However, plant growth conditions could modulate rhizomes' production as variation within varying stand densities were observed. Finally, we observed an increase of allocation to stem bases in older stands, coupled to a decrease of the reproductive allocation through lower proportion of reproductive tillers. Thus, work dedicated to understanding the allocation of resources in the plant will be beneficial to help identify possible synergies and trade-offs between grain production and ecological services.

1. Introduction

Perennial grains are currently being developed to meet some of the sustainability challenges facing agriculture. The expected benefits of using perennial grains include reduced soil erosion, nutrient conservation and increased carbon storage (DeHaan et al., 2023). Among the candidate species of perennial grasses being considered as a grain crop, the cool-season perennial grass *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey is one of the most promising species with significant advancements in germplasm development and agronomics (Bajgain et al., 2022). *Th. intermedium* can be grown to produce both grain for human consumption and forage for livestock. Alongside the production of these marketable outputs, the perenniality of this crop offers ecological benefits through the development of an extensive root system and a continuous soil cover (Ogle et al, 2011; Sainju et al., 2017; Duchene et al., 2020).

However, its allocation of resources to grains is currently low with a harvest index around 10 %, and a nitrogen (N) harvest index ranging from 20 to 50 % (Fagnant et al., 2024; Martensson et al., 2022). With low grain yield compared to annual counterparts, breeding efforts to improve grain production have been ongoing since the early 2000s (Bajgain et al., 2022; DeHaan et al., 2023). These efforts have resulted in advanced populations with larger seed size, reduced shattering, greater free-threshing, and lodging resistance. Yet important questions remain regarding a potential trade-off between grain yield potential and life span (Lundgren and Des Marais, 2020). Vico et al. (2016) highlighted an invariance of biomass allocation patterns of perennial crops under selection that maintained the large belowground allocation of wild perennials, and thus could maintain their multifunctionality. On the contrary, Gonzalez-Paleo et al. (2023) compared populations of *Silphium integrifolium* Michx. and suggested that populations that have undergone selection for high seed yield could imply higher external N inputs compared to wild accessions.

In annual grain crops, the pattern of assimilates partitioning changes with the transition from vegetative to reproductive development. In the latter stages of grain filling, there is usually an extensive remobilization of assimilates from stems and leaves to grains (Hay and Porter, 2006). In contrast, for long-term survivorship or under harsh survival conditions, perennial plants prioritize allocating assimilates to belowground and storage structures (e.g., roots, rhizomes, or stem bases) which allows them to conserve resources efficiently for later mobilization to other tissues (Roumet et al., 2006; Vico et al., 2016). As perennial grass, successful regrowth of *Th. intermedium* after cutting could depend upon different traits such as the activation of axillary meristems located in the bases of stems and requiring sufficient resources (Hendrickson and Olson, nd). *Th. intermedium* is also producing rhizomes, that are underground plant stems capable of producing the shoot and root systems of a new ramet (Sakiroglu et al., 2020).

Regarding plant N requirements, they are met by two principal methods: translocation from more mature tissues or storage organs and uptake of mineral N from soil solution (Woodmansee et al., 1981). As highlighted by Dobbratz et al. (2023), perennial grasses can translocate N across plant organs seasonally to conserve nutrients, but translocation occurs differently among species. In the study of Bausenwein et al. (2001), the aboveground N requirements of *Agrostis capillaris* L. and *Festuca rubra* L. in early spring were met by N remobilization from the old leaves (present on over-wintering tillers). In this study, no N remobilization from the root system to support aboveground growth was observed. In contrast, species such as *Panicum virgatum* L. or *Miscanthus* x *giganteus* make greater use of N translocation from rhizomes for spring tissue growth thanks to earlier storage during senescence at the end of the previous growth season (Dohleman et al., 2012). Finally, stem bases and roots can store C and N to ensure leaf development when photosynthetic activity is temporarily reduced by defoliation (grazing or mowing) (Lemaire, 2001), also varying from one species to another (Turner et al., 2007). For example, N reserves play a minor role in the regrowth of *Dactylis glomerata* L. after defoliation (Turner et al., 2006), while it plays a major role for *Lolium perenne* L. (Ourry et al., 1988). In the study of Turner et al. (2007) on *Bromus willdenowii* Kunth., stem bases represented the primary site for energy storage compared to roots, suggesting that this compartment should not be underestimated and overlooked in the shadow of root system studies.

Previous studies *on Th. intermedium* have provided information on the distribution of crop dry matter (DM) in both above- (Fagnant et al., 2023; Jungers et al., 2018) and belowground parts (Pugliese et al., 2019). Allocation of DM to roots to a depth of 60 cm ranged from 23 to 50 % of total DM (Rakkar et al., 2023), which was higher compared to annual counterparts (Sprunger et al., 2018; Duchene et al., 2020). As observed by Sakiroglu et al. (2020), rhizomes of *Th. intermedium* accounted for 17 % of the belowground DM in the first 10 cm of soil. With greater root DM and high water-soluble carbohydrates concentration within rhizomes, both rhizomes and roots played important roles in the survival and regrowth of *Th. intermedium* (Sakiroglu et al., 2020). However, a more pronounced investment in underground structures compared to annual crops presents a potential challenge for perennial grain crops in that diversion of resources away from seeds can limit grain yield (Vico et al., 2016).

In addition to the challenges and opportunities associated with biomass allocation trends of perennial grain crops, use and mobility of N between the different compartments also warrants special consideration. Sprunger et al. (2018) observed that *Th. intermedium* had a higher whole-crop N use efficiency than annual wheat. However, Fagnant et al. (2023) observed that the well-known translocation of N from senescent leaves and stems at the end of the growing season was not completely allocated to spikes. This resulted in a loss of 50 % of the N contained within the aboveground biomass (i.e., above 5 cm of the ground level). This was related to the low seed sink strength of the crop, which is associated with significant investment in perennial structures such as roots, rhizomes and stem bases as well as inherent changes in plant physiology related to its perenniality (Jaikumar et al., 2013).

Since much remains to be done to understand the dynamics of resource allocation in relation to *Th. intermedium*'s growth strategy, the objective of this work was to quantify the distribution of DM and N allocation in the different plant parts (i.e., leaves, stems, spikes, stem bases, rhizomes and roots) as the crop ages. To do so, a chronosequence approach was used, based on two different experimental sites established in 2019 and 2021. This characterization of N allocation across plant tissues over the growing season is essential to improve crop performance through breeding and agronomic management.

2. Materials and Methods

2.1 Field experiment and crop management

In this study we used two experimental sites with similar soil types located in the experimental farm of ULiège – Gembloux Agro-Bio Tech, Belgium, during the two cropping years of 2022 and 2023. The statistical design and management of the two experiments are synthetized in Table 1. Overall, the BE1 experimental site had only a generation factor studying 4 different generations from the breeding program of the Land Institute. The BE2 experimental site had a sowing date factor (main treatment, three contrasted sowing dates) and an interrow spacing factor (split-plot treatment, two interrow spacings). The different treatments weren't studied in this study and only one common treatment (detailed in Table 1) was selected at each site. For each experimental site, each plot (i.e., 4 x 8 m) was divided into two to obtain one plot dedicated to grain yield measurement in summer and the other one to destructive sampling during the growing season.

The two experimental sites are characterized by deep and fertile soil conditions with a clay-loam soil type, and optimal nutrients content. At the end of April 2022, the N content averaged 95 kg ha^{-1} for the BE1 site and 34 kg ha^{-1} for the BE2 site over the first 90 cm of the soil. The organic carbon content averaged 11 g kg^{-1} for the BE1 site and 15 g kg^{-1} for the BE2 site.

Some weather indicators were calculated to characterize the different cropping years for various development phases: vegetative and reproductive phase as described in Fagnant et al. (2024). Whitin these different development phases, the amount of rainfall, the number of dry days (i.e., where the rainfall was lower than 0.6 mm), the number of scalding days (i.e., where the maximum temperature was above 30 °C), the accumulation of solar radiation, the accumulation of growing degree days and finally the water balance (Ks coefficient) were calculated. Water balances were drawn up to assess soil water availability for each experimental site and to estimate the daily depletion of the extractable soil water (TAW) to a depth of 2 m as performed and described in Fagnant et al. (2024).

2.2 Aboveground and belowground samplings

Data was collected during the two cropping years of 2022 and 2023 (i.e., two grain production years), corresponding to the first and second cropping years of the BE1 site and the third and fourth cropping years of the BE2 site (Table 1). 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 beginning of stem elongation (BBCH30), the flag leaf (BBCH39), the flowering (BBCH65) and the grain maturity (BBCH89) stages as well as the vegetative stage during the autumn regrowth (BBCH2.). When needed, the four phenological stages were translated into development units (sum of degree-days corrected by photoperiodic and cold requirement effects) as proposed in the STICS soil-crop model and described in the study of Duchene et al. (2021). For the BBCH30 stage, they range from 178 to 314, for the BBCH39 stage from 464 to 501, for the BBCH65 stage from 925 to 1081 and for the BBCH89 stage from 1591 to 1837 units. Concerning the vegetative stage, its development unit stayed at 0 as cold and photoperiod requirements are completed after winter, as synthetized in supplementary material (Table S3).

Aboveground biomass was sampled within a 50 x 50 cm quadrat per plot, cut at 5 cm above the soil surface. Leaves were separated from stems, and spikes (when present) were cut just below the lowest spikelet. The different aboveground plant parts (i.e., leaves, stems and spikes) were weighed to obtain fresh matter and then oven-dried (72 h at 60 °C) and weighed to obtain DM. When collecting the aboveground biomass, the number of tillers and spikes during the reproductive phase (i.e., from BBCH30 to BBCH89 stage) were counted within the quadrat. Tiller fertility was calculated by dividing the number of spikes by the number of tillers at BBCH89 stage. The tiller mortality was also calculated by dividing the difference between the number of tillers at BBCH30 and BBCH89 stages by the number of tillers at BBCH30 stage. The leaf area index (LAI) considering the surface area of leaves and the green area index (GAI) considering the surface area of leaves and stems, were also measured at three phenological stages (BBCH30, BBCH39 and BBCH65) by collecting leaves and stems on 50 cm of a seeding line in one replicate. They were then laminated with transparent adhesive cover on paper sheets and scanned. These leaves and stems were weighed beforehand to estimate the specific leaf area (i.e., ratio of leaf area to leaf dry mass) and the specific leaf and stem area (i.e., ratio of the leaf and stem area to leaf and stem dry mass). The specific leaf area was used to estimate LAI, and the specific leaf and stem area was used to estimate GAI over the three other replicates.

After the aboveground biomass collection, the belowground and ground-level biomass were sampled within the same 50 x 50 cm quadrat at all phenological stages (i.e., from BBCH30 to BBCH2. stage). A total of four samples were collected from each quadrat; two were collected within the sown crop rows and two between the crop rows. Samplings were performed with a metallic probe of 12.5 cm in diameter to 15 cm depth. This sampling depth was chosen as it accounted for most of the biomass with the minimum sampling effort. As highlighted in other studies, most of the root biomass of perennial forages to a 1 m deep occur within the first 20 cm of the soil (Sainju et al., 2017), and Sakiroglu et al. (2020) observed that 79 % of root biomass and 95 % of rhizomes biomass of *Th. intermedium* were contained in the first 10 cm of the soil. The samples were stored at 4°C before the washing process which occurred within a maximum of 2 days after collection. The samples were cleaned with low water pressure over 1 mm sieve to remove soil particles. The samples were then oven-dried (72h at 60°C) and sorted to isolate roots, rhizomes, and stem bases. Stem bases represented the crowns and stem biomass located at the ground-level and up to 5cm above the soil surface, containing a part of the leaves at vegetative stages as illustrated in Figure 1. All the samples were then weighted to obtain DM. Finaly, the root mass fraction was calculated as the root DM divided by the total plant DM.

Table 1: Detailed information about the experimental sites, their design and management.

	Experimental sites	
Site code:	***BE1***	***BE2***
GPS Long. (DD)	4.7091	4.7052
GPS Lat. (DD)	50.5652	50.5659
Soil type:	Clay loam	
Climate:		
Average annual rainfall (mm)	852	
Average annual min temperature (°c)	7	
Average annual max temperature (°c)	14.2	
Type of experiment:	Randomized block design (4 replicates)	andomized split-plot design (4 replicates)
Implementation:		
Sowing date (DD/MM/YYYY)	09-09-2021	23-08-2019
Seed population	TLI-C5: 5th cycle of selection of The Land Institute, Kansas, USA	
Seeding rate	25 kg ha^{-1}: leading to a plant density of 73 plants m^{-2} in the first year	20 kg ha^{-1}: leading to a plant density of 366 plants m^{-2} in the first year
Interrow spacing (cm)	28	25
Field management:		
Nitrogen fertilization (kg N ha^{-1}) at:		
BBCH30	75	50
BBCH39	25	50
Autumn	50	50
Weeding	Chemical + mechanical	
Crop protection	/	
Post-harvest residue management	Chipping at 5 cm of the ground after grain harvest + Fall mowing	

Figure 1: Illustration of a clean and fresh sample of belowground and ground-level biomass of *Th. intermedium* from the BE1 experimental site (i.e., stem bases, rhizomes and roots).

N concentrations of all plant parts (i.e., leaves, stems, spikes, stem bases, rhizomes, and roots) were measured through the Dumas method (Dumas, 1831); N amounts were quantified individually for each replicate (across all experimental sites, cropping years and phenological stages).

At grain harvest, dedicated plots were harvested with a small plot combine harvester to obtain the grain yield on a cleaned, but unsorted seeds basis (i.e., a mix of hulled and dehulled seeds). This grain yield was divided by the total aboveground DM (i.e., leaves, stems, spikes and stem bases) at grain harvest to estimate the harvest index.

2.3 Data analysis

All data analyses were performed with R (R Core Team, 2024). Different mixed models were used with the *lmer* function from the *lme4* package. A first mixed model was created for variables measured at multiple phenological stages during the year, with the phenological stage and the experimental site considered as fixed factors, the crop age as a nested fixed factor within the experimental site and the block as a random factor. A second mixed model was created for variables only measured at grain maturity, with the experimental site considered as a fixed factor, the crop age as a nested fixed factor within the experimental site and the block as a random factor. Models were evaluated to ensure they met the assumptions of independence and normality of residuals through the *plotresid* function. These models were used in ANOVAs to analyze their influence on spike density, tiller density, DM and N amounts of the different plant parts (i.e., leaves, stems, spikes, stem bases, rhizomes and roots), root mass fraction, LAI and GAI, grain yield, harvest index, tiller fertility and tiller mortality. Following the ANOVAs, pairwise comparisons among treatment means were evaluated with the *emmeans* function from the *emmeans* package with a Tukey adjustment for multiple comparisons. If a significant interaction between fixed factors within the model was observed, this was considered in the post hoc test. Statistical significance was set at 0.05.

3. Results

3.1 Weather and soil water conditions

Weather and soil water conditions are presented in supplementary materials (Table S2). In 2022, rainfalls were low from BBCH30 to BBCH89 inducing a water stress from BBCH30 for the BE2 site and from BBCH39 for the BE1 site. The soil's water reserves were then slightly replenished the following autumn and winter, but not completely. Through low rainfall during the beginning of the spring 2023, water stress was also observed from the BBCH39 stage this year. In 2023, the accumulation of growing degree days was lower than in 2022.

3.2 Aboveground and belowground allocation of DM

The production and distribution of crop dry matter (DM) evolved with phenological development (Figure 2). Aboveground DM increased during the growing season, with maximum aboveground DM (i.e., composed of stems, leaves, and spikes) at grain harvest (i.e., BBCH89), ranging from 8 to 12 t of DM ha^{-1} as shown in supplementary materials (Figure S1). When the DM of stem bases (i.e., the biomass up to 5 cm above the ground) is included, the total aboveground DM ranged from 13 to 21 t of DM ha^{-1} (Figure S1). During the growing season, the highest DM of leaves was observed at the flag leaf stage (i.e., BBCH39; p-value<0.001), corresponding to the maximal photosynthetic leaf area (i.e., LAI) (pvalue<0.05; Table 2). The highest DM of stems and stem bases was observed at grain harvest, where it represented 82 % of the aboveground DM (p-value<0.01; Figure 2). The GAI (i.e., representing the green area of leaves and stems) was the highest at the flowering stage in 2022 and at the flag leaf stage in 2023 (p-value<0.001; Table 2).

Regarding belowground DM (i.e., roots and rhizomes) within the first 15 cm of the soil, it ranged from 2 to 7 t of DM ha^{-1} (Figure S1). The effect of phenological development on root DM was only significant in 2022, with a higher DM at the end of the growing season (i.e., BBCH65 or BBCH89; p-value<0.001; Figure S1). As observed in Table 2, the root mass fraction (i.e., root DM divided by the total plant DM) ranged from 14 to 42 %, with the highest value observed during vegetative stages (i.e., BBCH30 and BBCH2.; p-value<0.001; Table 2). The DM of rhizomes represented between 4 to 22 % of the belowground DM and was not influenced by phenological stages except in the first cropping year of the BE1 site. Indeed, the initiation of rhizomes appeared between the flag leaf and the flowering stages that increased their DM during the first growing season (p-value<0.001; Figure 2A).

Concerning grain yield components, the tiller density at the beginning of the growing season ranged from 349 to 1086 tillers m^{-2}, tiller fertility ranged from 66 to 95 % and the final number of spikes ranged from 377 to 603 spikes m^{-2} (Table 2). The spikes represented around 10 % of the aboveground DM (comprising stem bases). This resulted in a grain yield ranging from 0.9 to 1.7 t ha^{-1} and a harvest index from 5 to 10 % of the aboveground DM comprising the stem bases (Table 2).

The experimental site also induced some differences in the production and distribution of crop DM at some phenological stages. Globally, a higher stem and root DM was observed for the BE2 site compared to the BE1 site (p-value<0.01; Figure 2). On the other hand, the BE1 site had higher rhizomes DM (p-value<0.001; Figure 2), representing 20 % of the belowground DM compared to 8% for the BE2 site.

An influence of crop age, specific to the two experimental sites, was observed. At the BE1 site, aboveground DM, LAI, and GAI increased from the first to the second cropping year (p-value<0.05; Figure S1; Table 2). In parallel, the tiller density increased until the beginning of the second reproductive phase (p-value<0.001), resulting in a higher spike density and grain yield in the second year (p<0.05; Table 2). In contrast, allocation of DM to spikes was similar between the two years of the BE1 site (Figure S1). During the first cropping year, we observed an increase of the belowground DM (i.e., roots and rhizomes) inducing higher belowground DM at the beginning of the second cropping year (p-value<0.001; Figure S1).

Focusing on the BE2 experimental site, the crop was well established and no significant differences of DM (both above- and belowground) and photosynthetic area were observed between cropping years. An exception can be mentioned with stem bases DM that increased from the third to the fourth cropping year (p-value<0.05; Figure S1). In parallel, a strong decrease of grain yield was observed between these two cropping years (p-value<0.001; Table 2), as the harvest index dropped from 10 to 5 % and tiller fertility from 85 to 66 % (Table 2).

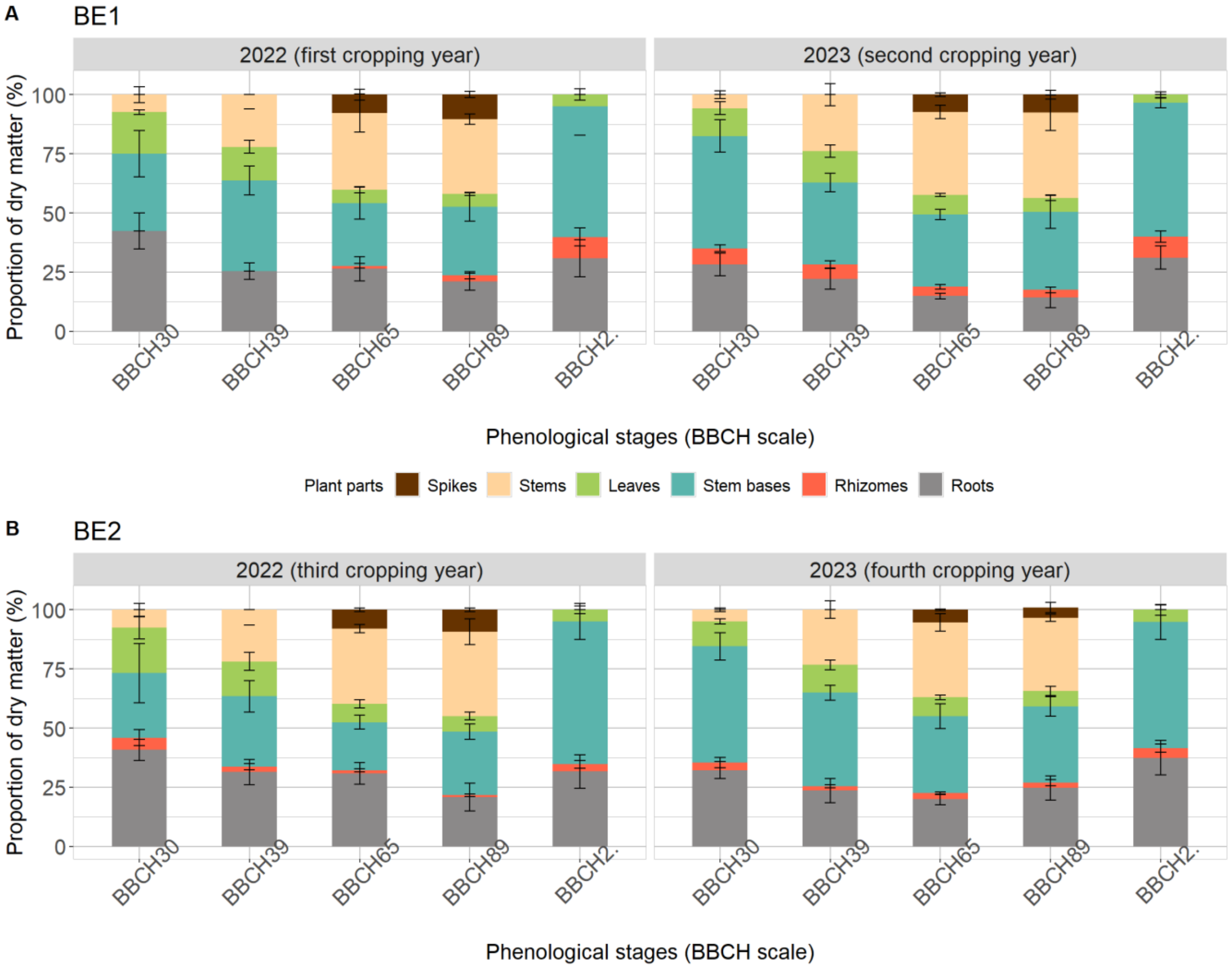

Figure 2: Dry matter allocation within *Th. intermedium* plant parts in relative proportion within the whole plant. (A) for the BE1 experimental site (i.e., first in 2022 and second cropping years in 2023) and (B) for the BE2 experimental site (i.e., third in 2022 and fourth cropping years in 2023). Error bars indicate the mean ± standard error.

Table 2: Mean (± standard error) grain yield, harvest index, spike and tiller density, leaf area index, green area index, root mass fraction of *Th. intermedium* for the BE1 and BE2 experimental sites in 2022 and 2023.

		BE1		BE2	
		2022	**2023**	**2022**	**2023**
		(First cropping year)	(Second cropping year)	(Third cropping year)	(Fourth cropping year)
Grain yield (t ha^{-1})		1.0±0.1 A	1.3±0.1 B	1.7±0.1 B	0.9±0.1 A
Harvest index (%)		7±2	7±1	10±1 B	5±2 A
Spike density (spikes m^{-2})		377±38 A	603±38 B	588±74	545±74
Tiller density	BBCH30	349±42 A	1066±61 B		1086±64 b
(tillers m^{-2})		ab	b	1053±111	ab
	BBCH39	335±42 A	826±61 B		689±64
		ab	ab	828±111	a
	BBCH65	322±42 A	644±61 B		819±64
		a	a	960±111	ab
	BBCH89	511±42 A	635±61 B		820±64 a
		b	a	692±111	b
Leaf Area Index	BBCH30	1.0±0.2 A	2.6±0.3 B	3.0±0.4	2.9±0.2
			b		b
	BBCH39	1.3±0.2 A	4.3±0.3 B	2.7±0.4 A	4.5±0.2 B
			c		c
	BBCH65	0.9±0.2 B	0.3±0.3 A	1.5±0.4 B	0.5±0.2 A
			a		a
Green Area Index	BBCH30	1.2±0.6 A	3.2±0.3 B	3.8±1.0	3.7±0.4
		a	a	a	c
	BBCH39	2.4±0.6 A	8.4±0.3 B	4.1±1.0 A	8.1±0.4 B
		ab	b	a	a
	BBCH65	3.9±0.6 A	6.8±0.3 B	10.3±1.0 B	5.5±0.4 A
		b	b	b	b

Root mass fraction	BBCH30	0.42±0.03 B c	0.28±0.02 A bc	0.41±0.03 b	0.32±0.03 bc
	BBCH39	0.25±0.03 ab	0.22±0.02 ab	0.31±0.03 B ab	0.24±0.03 A ab
	BBCH65	0.26±0.03 B ab	0.15±0.02 A a	0.31±0.03 B ab	0.2±0.03 A a
	BBCH89	0.21±0.03 a	0.14±0.02 a	0.21±0.03 a	0.25±0.03 a
	BBCH2.	0.31±0.03 b	0.31±0.02 c	0.32±0.03 ab	0.38±0.03 c

Note. Means sharing the same letter are similar or having no letter (*p-value*<0.05). Lowercase letters represent the result of the post hoc analysis of the effect of phenological stage and uppercase letters represent the result of the post hoc analysis of the effect of the crop age within each experimental site.

3.3 Aboveground and belowground allocation of N

As observed for DM, N distribution in the crop evolves with phenological development. N in the aboveground biomass (i.e., leaves, stems, spikes and stem bases) represented 54 to 77 % of the total N amount of the plant, with the lowest value observed at the fall vegetative stage (i.e., BBCH2.; Figure 3). The amount of N within leaves was maximal at the beginning of the growing season (i.e., BBCH30 and BBCH39). In stems, the amount of N globally increased until the flowering stage (i.e., BBCH65) before decreasing until grain maturity (i.e., BBCH89; p-value<0.05). However, the amount of N in the spikes did not increase after flowering (Figure 3). Spikes represented 14 to 35 % of the aboveground N amount (stem bases included) while stem bases represented 25 to 89 %. When an effect of phenological stages was observed (i.e., in 2023; p-value<0.01), the N amount of stem bases decreased from the beginning of stem elongation (i.e., BBCH30) to the flag leaf (i.e., BBCH39) stage. An increase was then observed at grain maturity (i.e., BBCH89), reaching the highest level at the fall vegetative stage (i.e., BBCH2.; Figure 3).

N allocation within the belowground biomass (i.e., roots and rhizomes) was mainly directed to roots, which accounted for 71 to 97 % of the belowground N amount (Figure 3). When an effect of the phenological stage was observed, the amount of N in rhizomes was the highest at the fall vegetative stage (i.e., in 2023 for the BE2 site and 2022 for the BE1 site; p-value<0.05). Within roots, the amount of N was the highest at the end of the growing season (i.e., flowering or grain maturity in the BE2 site; p-value<0.01; Figure 3).

Considering the total amount of N in the plant (i.e., above- and belowground), it decreased from the flag leaf or the flowering stage (i.e., BBCH39 and BBCH65) until the grain harvest for the site BE2 in 2022 (i.e., loss of about 50 kg of N ha^{-1}) and for the site BE1 in 2023 (i.e., loss of about 30 kg of N ha^{-1}) (Figure 3). Indeed, a decrease of N in leaves, stems, and roots was observed without any increase in spike N amounts.

The distribution of crop N was influenced by the experimental site. The BE2 site had a higher amount of N in stem bases and roots compared to the BE1 site (p-value<0.01; Figure 3). The amount of N in rhizomes was higher for the BE1 site (p-value<0.001). A difference was also observed in 2022, corresponding to the first cropping year of the BE1 site and the third cropping year of the BE2 site, with a higher amount of N in leaves at the beginning of the growing season and in stems for the BE2 site (p-value<0.001; Figure 3B).

The crop age nested within the two experimental sites also influenced the crop N. For BE1 experimental site, the amount of N in leaves, stem bases, roots and rhizomes increased from the first to the second cropping year (p-value<0.05; Figure 3A). The amount of N within spikes was similar between the first and the second year (Figure 3A), although its proportion within the aboveground N amount (comprising stem bases) decreased from 35 to 21 % in the second cropping year. In BE2 experimental site, with plants in their third and fourth cropping years, the crop age had no effect on the amount of N in stems, leaves and roots. However, a higher amount of N within the stem bases was observed in the fourth cropping year, coupled with a lower amount of N within the spikes (p-value<0.01; Figure 3B). The amount of N in rhizomes was also higher in the fourth cropping year, but only for the flowering stage (p-value<0.05; Figure 3B). The highest amount of N in the whole plant corresponded to the time of the highest observed root DM (Figures S1 and 3). Conversely, the decrease in root DM at the end of the growing season (Figure S1, BE2-2022 and BE1-2023) led to a decrease in the total amount of N in the plant from 34 to 56 kg of N ha^{-1}.

The average loss of nitrogen from the field by export of leaves, stems and spikes during summer harvest was calculated and reached 60 kg of N ha$^{-1.}$ This represented 55 % of the total N amount during the first cropping year and 30 to 39 % in regrowth years (Figure 3).

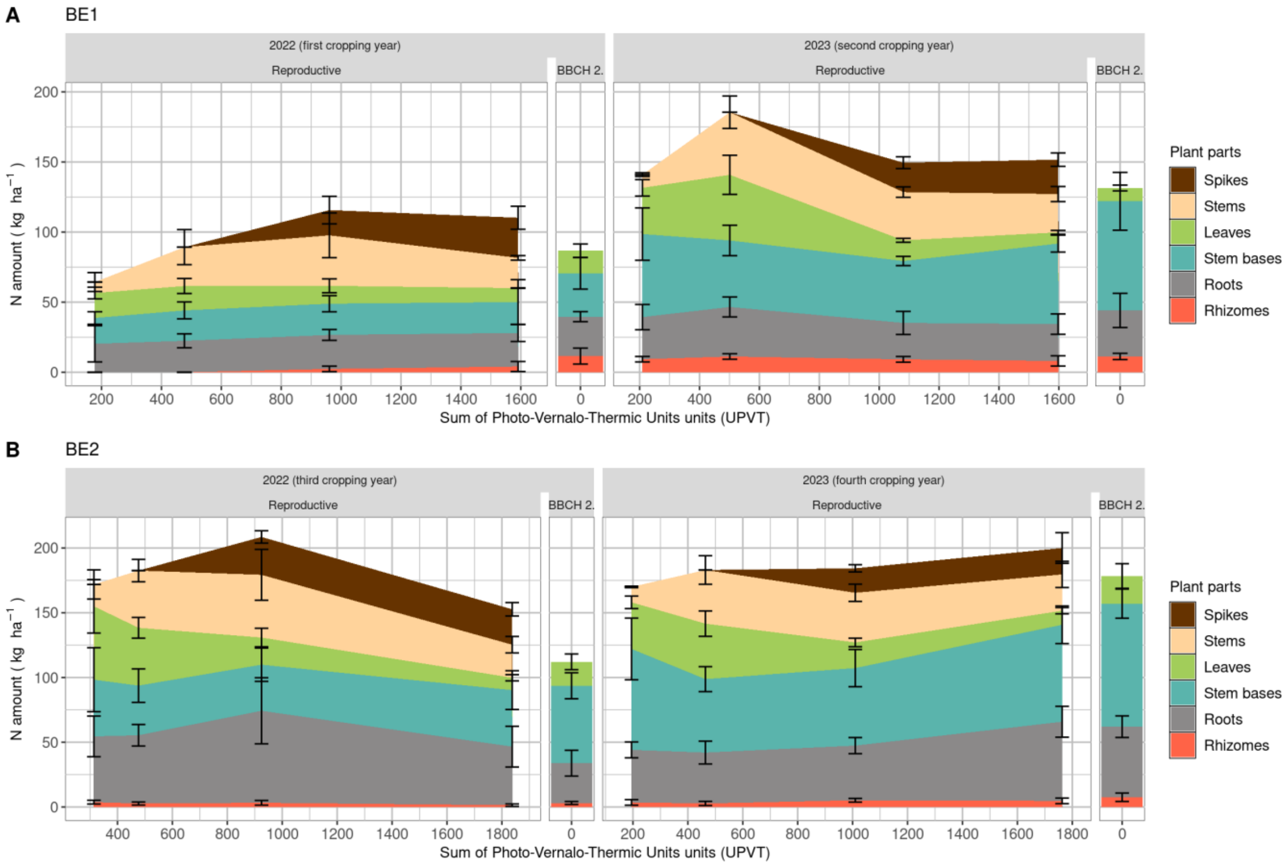

Figure 3: Nitrogen (N) allocation within Th. intermedium plant parts (A) for the BE1 experimental site (i.e., first in 2022 and second cropping years in 2023) and (B) BE2 experimental site (i.e., third in 2022 and fourth cropping years in 2023). Values

are presented in order of sampling but divided in two development phases, the vegetative phase of autumn before floral induction and the reproductive phase after the induction (Duchene et al. 2021). X-axis represents accumulation of photo-vernalo-thermic units. Black error bars represent the mean ± standard error.

4. Discussion

4.1 Conserving resources for clonal development

As a cespitose grass with short rhizomes, the stem bases of *Th. intermedium* contain belowground buds for the production of intra- and extravaginal tillers (Herben et al., 1994; Lafarge and Durand, 2011). It represented an important part of the plant DM and N stocks (i.e., up to 9 t of DM ha^{-1} and 95 kg of N ha^{-1} for regrowth years) and increased with aging plants (Figures S1 and 3).

Within a given growing season, the DM of stem bases remained relatively constant, although it generally peaked at grain harvest. This increase of DM in stem bases may correspond to an increase in water-soluble carbohydrate reserves, as also observed in the basal internodes of *Lolium perenne* (Trethewey and Rolston, 2009). Meanwhile, the increase in the amount of N in stem bases after flowering until the next fall vegetative stage (Figure 3) suggests that stem bases could be a sink for translocated N from leaves, stems or roots, or allocation of new N assimilated from the soil pool. Unlike the stem bases, there was no significant increase in the N amount of roots and rhizomes after flowering (Figure 3). This resource allocation dynamic was consistent with the literature on perennial grasses, which suggests an accumulation of resources in the stem bases at the end of the growing season, since this is where the intercalary meristems, responsible for the expansion of new leaves, stems, and ramets for the next season are located (Lemaire, 2001; Yang and Udvardi, 2018).

Unlike stem bases, rhizomes (i.e., extravaginal tillers) represented a low and constant proportion of the total DM and N of the plant, from 1 to 12 kg of N ha^{-1} (Figures 2 and 3). Whether this proportion of rhizomes is due to a dynamic balance of mortality and new production or to the persistence of the first emitted rhizomes remains to be studied. These rhizomes were initiated between the flag leaf and the flowering stage of the first cropping year, resulting in vegetative tillers during this first year. Herben et al. (1994) also observed that rhizomes of *Festuca rubra* developed later in the growing season compared to intravaginal tillers that develop sylleptically (i.e., at the same time as the growing buds). Thus, the closely spaced ramet produced by *Th. intermedium* could allow a better use of locally abundant resources (Bam et al., 2023). This growth strategy is also called 'phalanx', as introduced by Doust (1981), in contrast to the 'guerilla' strategy that produces long spacing organs (i.e., stolons or rhizomes) to explore and spread new ramets.

When the crop was in its first two cropping years (i.e., BE1 site), a higher proportion of rhizomes within the belowground biomass was observed, averaging 20 % compared to only 8 % for older stands (site BE2). This could be the result of modulation driven by plant growth conditions. In our study, the BE1 site was characterized by a low plant and tiller density (i.e., mean of 380 tillers m^{-2} in 2022; Table 2) inducing a lower intraspecific competition. This may have favored tiller production (i.e., either intra- or extravaginals) thanks to better light penetration within the canopy (Langer, 1963). The high rhizome production rate in year one likely resulted in vegetative recruitment as evidenced by the higher tiller proportion in year two at the BE1 site. Some plasticity in growth form under different environmental conditions was also observed in other perennial grasses (Bam et al., 2023; Hartnett, 1993). In Bam et al. (2023), *Bromus inermis* Leyss. and *Pascopyrum smithii* (Rydb.) Á.Löve were able to switch to different proportions of extravaginal tillers under different competitive conditions. The ability of *Th. intermedium* to alter its bud placement to favor intra- or extravaginal tiller recruitment in response to competitive evolution would benefit from further studies.

4.2 Reproductive allocation

The values of the harvest index ranged from 9 to 15 % resulting in a grain yield from 0.9 to 1.7 t ha^{-1} (Table 2), which is in the upper range of values known for *Th. intermedium* and even higher than those observed in our pedoclimatic conditions (Fagnant et al., 2024). This could be explained by the relatively high tiller fertility, which ranged from 66 to 95 % (Table 2), compared to Fagnant et al. (2024) where it ranged from 45 to 84 %. As mentioned by Fernandez et al. (2021), tiller fertility of *Th. intermedium* explained the largest variation in grain yields. In our study, this high tiller fertility may be explained by the optimal tiller density observed at the beginning of the growing season, which did not exceed 1086 tillers m^{-2} (Table 2), reducing competition between tillers within the stand (Fagnant et al., 2024).

In absolute terms, DM and N allocated to spikes remained similar for different crop stand ages, with an average of 25 kg of N ha^{-1} for a DM of 1.8 t of ha^{-1} at grain maturity (Figures 2 and 3). However, the proportion of N allocated to spikes changed with stand age. At grain maturity, spikes contained 26 % of the total N in the first cropping year (BE1-2022) compared to maximum 18 % in regrowth years (Figure 3). This was mainly explained by the lower N allocation to stem bases in the first cropping year (i.e., 20 % of the total N amount at BCBH89, against up to 39 % in regrowth years). Proportionally, the sexual reproductive effort within an establishing crop was higher.

However, these constant allocations to spikes weren't translated into constant grain yield across stand ages. An increase of grain yield was observed from the first to the second cropping years of the BE1 site while a sharp decline of the grain yield was observed between the third and the fourth cropping years of the BE2 site (i.e., loss of 50 %). These trends could be related to the production of vegetative tillers, reducing the tiller fertility and the grain yield for these years (Table 2). Indeed, during the first year of the BE1 site, vegetative tiller recruitment was observed while a lower tiller mortality coupled with a thickening of stem bases at the end of the fourth growing season was observed for the BE2 site. The higher proportion of vegetative tillers had likely diverted resources away from the growing spikes (Yang et al., 2019) and probably prevented floret utilization and spike's filling. In *Lolium perenne* some reports suggest that younger daughter tillers may compete with seeds for resources during reproductive development (Trethewey and Rolston, 2009). Overall, the results of this study suggest that the perenniality of yields in *Th. intermedium* can be very variable from one situation to another, depending on the development dynamics of tiller cohorts and resource conditions. As suggested by Altendorf et al. (2021), selection for grain yield improvement should reduce excessive tillering to increase the yield per spike, potentially involving a reduced forage potential through lower vegetative production.

Compared with annual crops, the low grain yields of *Th. intermedium* are also associated with low mobilization of resources to the spikes and grains after flowering that didn't statistically increase (Figure S1 and 3). As explained by Altendorf et al. (2021), opportunities to produce seeds are plentiful in *Th. intermedium*, but relatively few are utilized. The importance of floret site utilization on yield per spike would suggest that it should be a major target of selection. In addition, a limited N storage capacity of shoots (i.e., leaves and stems) of *Th. intermedium* was observed, that reached a maximum of 92 kg of N ha^{-1}, with a LAI ranging from 3 to 4.5 at the flag leaf stage (Figure 3, Table 2). In contrast and under unstressed conditions, cereals such as wheat or rice can develop a high LAI (i.e., from 7 to 8), which allows a large capacity to store organic N compounds in shoots (up to 250 kg N ha^{-1} at flowering in wheat; Gastal et al. (2015)). After flowering, nutrients are remobilized from leaves and stems and are used primarily for grain production, ensuring maximum reproductive success (Yang and Udvardi, 2018). For these annual species, 60 to 95 % of grain N at harvest comes from remobilization of N stored in roots and shoots prior to flowering (Gastal et al., 2015).

Future research on *Th. intermedium* will need to identify the ways in which increased resource allocation in the grain could be achieved: either by greater remobilization of resources to the spikes at the expense of other vegetative organs, or by mobilizing additional resources. In this context, Gonzalez-Paleo et al. (2023) compared populations of *Silphium integrifolium* Michx. and observed that improved populations had greater N allocation to leaves and seeds coupled with a lower storage of N in stem bases, which could imply higher external N inputs compared to wild accessions.

4.3 Root growth and activity drive the N balance of the crop

Roots within the first 15 cm of the soil accounted for a significant proportion of plant DM throughout the growing season, which was also observed in other studies on *Th. intermedium* (Duchene et al., 2020). During the establishment year, an increase of the belowground biomass was observed (Figure S1A) as most of the new root growth occurred in the first year (Woeltjen et al., 2024b). Unfortunately, the depth at which we sampled belowground organs didn't account for all the root biomass. Nevertheless, this sampling depth should at least represent between 50 and 80 % of the produced belowground biomass as observed in other studies (Sainju et al., 2017: Duchene et al., 2020; Sakiroglu et al., 2020).

The roots N content didn't significantly vary with stand age and was comprised between 20 and 70 kg of N ha^{-1}. However, our sampling methodology could have led to an underestimation of real belowground nitrogen dynamics due to limitations in depth samplings. In a study that sampled root biomass up to 90 cm, researchers measured from 8 to 32 kg of N ha^{-1}, and this pool was usually two times greater in older stands and largely driven by variations of root biomass instead of root tissue N concentration (Dobbratz et al., 2023). Other studies have also reported a larger root N pool in second year stands (Woeltjen et al., 2024a). As root production is directly linked to the construction of the tiller (Lafarge and Durand, 2011), future studies will need to identify acceptable trade-offs between managing grain yield over time by limiting excessive tillering activity and root production and accumulation of belowground N pool.

At the end of the growing season, a loss of around 25 kg of N ha^{-1} could be observed and was not explained by the reduction in the root system. We believe that this N could have entered the soil system through rhizodeposition. To support this hypothesis, Dobbratz et al. (2023) observed an increase in total soil N from the grain maturity to the fall regrowth under *Th. intermedium* crop. As they supposed, this influx could be due to root turnover and to the release of N-rich root exudates, or to a decreased amount of plant or microbial N immobilization relative to microbial N mineralization. Apart from root turnover, plant roots release inorganic and organic compounds by exudation from living roots and soil abrasion of the root cap protecting the meristematic zone (i.e., rhizodeposition). In their review, Wichern et al. (2008) calculated that wheat plants leave on average about 68 kg N ha^{-1} in the soil, with the rhizodeposition contributing to 26 kg N ha^{-1}. This rhizodeposition can stimulate soil microorganisms by transferring energy from plant roots, thereby increasing nutrient uptake through enhanced decomposition of soil organic matter.

The calculated export of nitrogen by the removal of aerial organs (Figure 3) is consistent with Dobbratz et al. (2023) who estimated that 40 to 75 kg of N ha^{-1} is exported each year during harvest. Thus, the remaining N (i.e., from 50 to 146 kg of N ha^{-1}), not exported and allocated to the perennating organs, was not valorized through marketable output, but was indirectly valued in the system by promoting strategies for survival and regrowth of the plant, which ultimately allow the production of ecosystem services (N recycling, soil protection, C storage, etc.).

5. Conclusion

Through a relatively low production of rhizomes, *Th. intermedium* is characterized by a conservative phalanx growth strategy, which is known to allow clonal plants to tolerate more stressful conditions and to make better use of locally abundant resources. High root production and the multiplication and thickening of stem bases over the years represent significant N sinks, especially at the end of the growing season and during vegetative stages. These N sinks are highly valuable functional structures to ensure perenniality and ecological services but could also limit the quantitative-qualitative optimization of the harvested products. As the reproductive effort of the crop is still low (biomass of spike and grains), future breeding progress for grain production will need to be informed by new work dedicated to understanding the allocation of resources in the plant to help identify possible synergies and trade-offs between the provisioning (export) and the supporting and regulating ecosystem services objectives.

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8. Supplementary materials

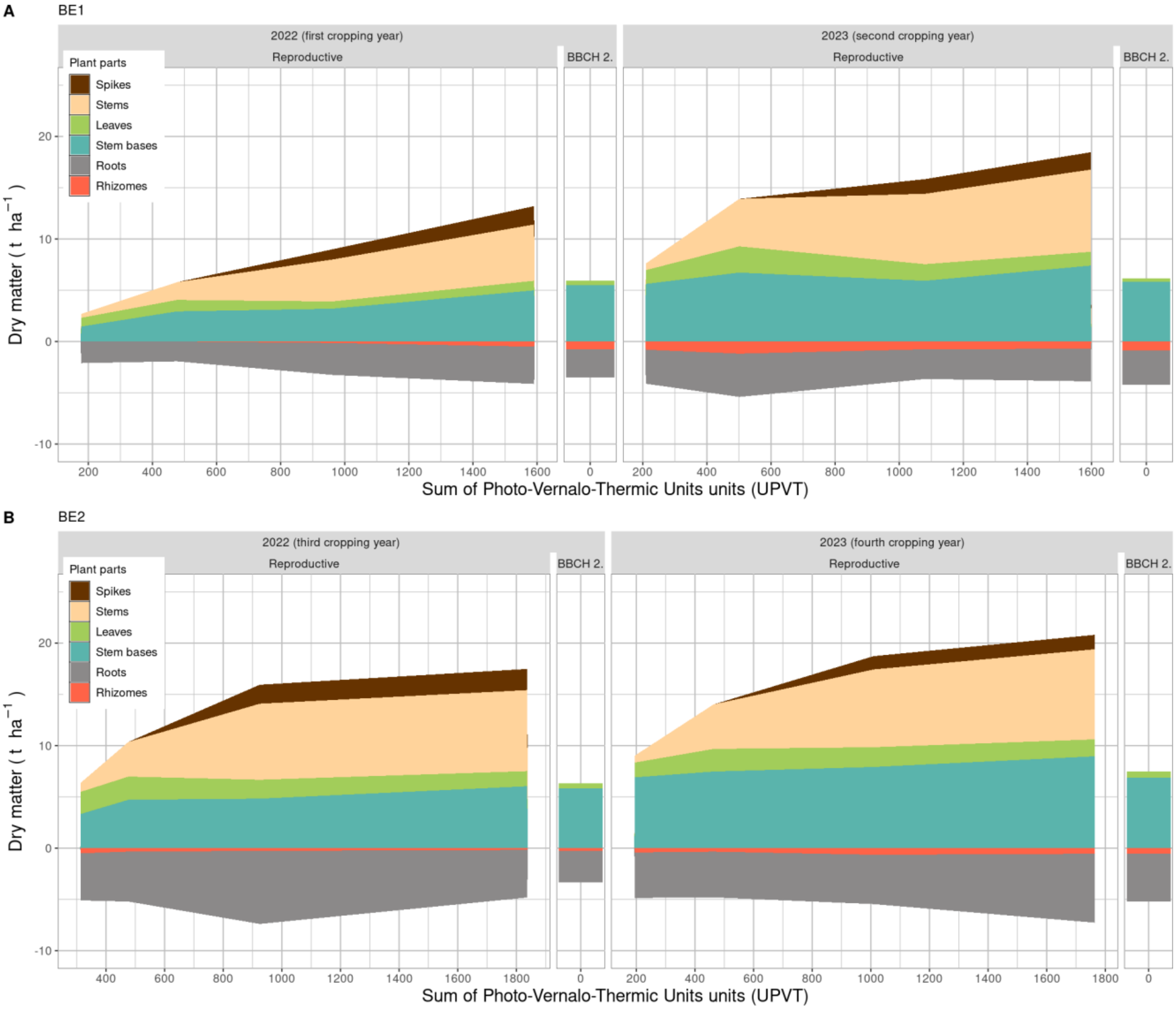

Figure S1 : Dry matter allocation within Th. intermedium plant parts (A) for the BE1 experimental site (i.e., first in 2022 and second cropping years in 2023) and (B) BE2 experimental site (i.e., third in 2022 and fourth cropping years in 2023). Dry matter values are presented in order of sampling but divided in two development phases, the vegetative phase of autumn before floral induction and the reproductive phase after the induction (Duchene et al. 2021). X-axis represents accumulation of photo-vernalo-thermic units. Belowground plant parts, roots and rhizomes' DM are expressed in a negative value range.

Table S1: Significance levels from the two-way and three-way ANOVA.

	DM						Root mass fraction	GAI	LAI	Tiller density	Tiller fertility	Spike density	Grain yield	Harvest index	N content					
	Leaves	Stems	Stem bases	Spikes	Roots	Rhizomes									leaves	Stems	Stem bases	Spikes	Roots	Rhizomes
Stage (S)	***	***	*	*	**	**	***	***	***	***	/	*	/	/	***	***	***	/		***
Experimental site (E)	***	***	*		***	***	*	***	***	***		***			***		***		***	***
Year (Y)	***	***	***			***	***	***	***	***			**	**			***			***
S*E		*			*	**						*	/	/	**			/		*
S*Y	*	**			**	*	***	***	***	*	***		/	/	*			/	**	
E*Y	***	**		**		***		***	***	***		***	***	**	***					
S*E*Y	**				**	***		**		**			/	/	***			/	***	*

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

Table S2 : Weather indicators of the experimental site during the two cropping years of the BE1 and BE2 sites for the different development phases. (A) the first vegetative phase from the seeding or from the estimation of total DM at autumn regrowth until BBCH30 stage. (B) the first reproductive phase from BBCH30 to BBCH39 stage, (C) the second reproductive phase from BBCH39 to BBCH65 stage, (D) the last reproductive phase from BBCH65 to BBCH89 stage and (E) the last vegetative phase from BBCH89 until the estimation of total DM at autumn regrowth.

Periods	**Rainfall [mm]**	**Dry days [days]**	**Scalding days [days]**	**Radiation [J/cm^2]**	**Growing degree days [°]**	**Ks Coefficient (BE1 site)**	**Ks Coefficient (BE2 site)**
2022(A) Sowing or BBCH2. to BBCH30	312	150	0	157752.5	746.15	1	0.92
(B) BBCH30 to BBCH39	21.1	27	0	66441.9	171.6	0.61	0.31
(C) BBCH39 to BBCH65	73.8	23	1	76217.8	171.85	0.02	0.02
(D) BBCH65 to BBCH89	32.9	40	4	105576.4	264.45	0	0
(E) BBCH89 to BBCH2.	133.3	49	7	100679.6	328.75	0.08	0.08
2023(A) BBCH2.-30	322.6	115	0	124339.3	534.05	0.53	0.52
(B) BBCH30 to BBCH39	45.7	25	0	67898.9	142.95	0.61	0.61
(C) BBCH39 to BBCH65	37.6	29	2	86553.2	186.45	0.02	0.02
(D) BBCH65 to BBCH89	116.2	18	1	73897.7	169.9	0.07	0.07
(E) BBCH89 to BBCH2.	131.7	53	4	102299.7	338.05	0.06	0.06

Table S3: Sum of development units (i.e., sum of degree-days corrected by photoperiodic and cold requirement effects, UPVT) for each experimental site and cropping year.

Year	**Stage**	**UPVT units BE1 site**	**UPVT BE2 site**
2022	BBCH30	177.6	314.2
	BBCH39	476.6	476.6
	BBCH65	960.9	924.5
	BBCH89	1591	1837.3
	BBCH2.	0	0
2023	BBCH30	209.3	194.9
	BBCH39	501.1	463.9
	BBCH65	1080.5	1009.7
	BBCH89	1597.5	1763.0
	BBCH2.	0	0