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Evaluation et optimisation agronomique des services de production d'une céréale pérenne, *Thinopyrum intermedium*.

Laura Fagnant

COMMUNAUTÉ FRANÇAISE DE BELGIQUE
UNIVERSITÉ DE LIÈGE - GEMBLOUX AGRO-BIO TECH

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Promoteurs :
Pr. Benjamin Dumont & Pr. Jérôme Bindelle
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Abstract

Thinopyrum intermedium (Host) Barkworth & D.R. Dewey is a C3 grass currently proposed as a dual-use perennial cereal, producing both forage and grains for human consumption. Its integration into agricultural systems offers various environmental benefits such as the soil protection or the regulation of the water quality, thanks to its permanent soil cover and its extented belowground activity. The species is in the nascent stages of domestication and its grain yield potential is still low compared to annual counterparts. Forage valorization could be a way of generating additional agricultural production. In order to optimize yields, a good description of its functional traits as well as a better understanding of its resource allocation strategies are essential. Within the experimental farm of the faculty of Uliège - Gembloux AgroBio- Tech, *Th. intermedium* was tested in field from 2017 to 2023 under two different valorizations : dual-use for grain and forage and simple use for forage production. To develop an adapted agronomic management to this new species, multiple practices were conducted (i.e., N fertilization, establishment conditions, association with legumes or mowing regimes). The species was also grown in a growth chamber during its first days of development and compared with winter wheat (*Triticum aestivum*).

Th. intermedium showed some environmental benefits compared with annual cereals, which may be associated with a significant investment in its long-lived organs such as its root system and stem bases. Relatively high amounts of biomass were produced with little N, as evidenced by its critical N dilution curve. In addition, low N export at the end of the growing season was observed (i.e., on average 60 kg of N ha^{-1}). The plant also showed some resource conservation traits, with a higher root tissue density and a preferential investment in root construction in the establishment phase. This latter trait may explain its relatively lower growth rate compared with annual cereals such as winter wheat. Finally, its cespiteous form, producing closely spaced ramets, induces a slow and radial spread. This development strategy may provide to the species a good tolerance to stressful conditions and an efficient use of local resources (i.e., monopolization strategy). However, its slow establishment phase requires new solutions to limit competition from desired or undesired plants within the field.

A certain compromise between vegetative growth, implying sufficient investment in long-lived organs, and grain production was observed. In fact, excessive biomass production and tiller density at the beginning of the growing season had a negative impact on the yield per spike. Although grain production is currently low (i.e., with a mean of 1 t ha^{-1}), a major finding was that it remained constant over four years of cultivation, which was never observed in other studies. Within our pedoclimatic conditions (relatively favourable to its crop development), this grain yield was explained by stable tiller and spike fertility. The optimal time for tiller initiation has yet to be determined, but it

should be early. Relatively dense sowing in late summer maximized tiller fertility. In regrowing years, overproduction of tillers and spikes should be avoided by a shredding of the post-harvest crop residues at ground level and a defoliation in autumn. This can also prevent light obstruction at the stem bases. Finally, an autumn fertilization of 50 kg of N ha^{-1} combined with an early spring fertilization of 50 kg of N ha^{-1} were effective in supporting tiller fertility while limiting the risk of excessive biomass.

The species was not suited to intensive forage management. In fact, the initiation of regrowth was weak when the apical meristems were cut during the stem elongation phase. Forage management seemed only relevant in a context of a dual grain-forage valorization. Spring and autumn mowing were characterized by low forage yields (i.e., maximum of 2 t of DM ha^{-1}), but with a good forage quality close to that of a fresh grass. Crop residue harvested at the time of grain maturity was characterized by good forage yields (i.e., 5 to 15 t of DM ha^{-1}), but with a low forage quality, close to that of straws. In the context of a dual valorization, spring mowing should be avoided, as it reduced grain yield, while autumn regrowth was generally too weak to justify a mowing. The association with a forage legume could therefore be interesting in order to increase production during the autumn period.

Integrating perennial cereals into agroecosystems therefore requires a rethinking of management strategies, and genetic selection remains crucial to achieve that goal. The latter should help to create new cultivars adapted to the different production contexts of farmers interested in establishing this new type of crop.

Résumé

Thinopyrum intermedium (Host) Barkworth & D.R. Dewey est une graminée en C3 actuellement proposée comme céréale pérenne à double usage, produisant à la fois du fourrage et des grains destinés à l'alimentation humaine. Son intégration dans les systèmes agricoles offre des services environnementaux variés tels que la protection des sols ou la régulation de la qualité de l'eau grâce à sa couverture permanente du sol et son activité souterraine étendue. La domestication récente de l'espèce entraine un rendement en grains actuellement faible par rapport à ses homologues annuels. La valorisation fourragère serait donc une voie pour générer une production agricole additionnelle. Afin d'optimiser les multiples productions de la culture, la description de ses traits fonctionnels et une meilleure connaissance de ses stratégies d'allocation des ressources sont essentielles. Au sein de la ferme expérimentale de la faculté de l'Uliège - Gembloux AgroBio- Tech, *Th. intermedium* a été testée au champ de 2017 à 2023, tant sous des valorisations double usage grain-fourrage que sous simple usage fourrager. Dans le but d'élaborer une conduite agronomique adaptée à cette nouvelle espèce, diverses modalités de gestions ont été menées (i.e., fertilisation azotée, conditions d'implantation, association aux légumineuses ou encore régime de fauches). L'espèce a également été cultivée en chambre de culture lors de ses premiers jours de développement et comparée au blé tendre d'hiver (*Triticum aestivum).*

Th. intermedium a montré certains bénéfices environnementaux par rapport aux céréales annuelles qui peuvent être associés à un investissement important dans ses organes permanents tels que la base des tiges et le système racinaire. Des quantités relativement élevées de biomasse aérienne ont pu être produites avec peu d'azote (N), comme en a témoigné sa courbe critique de dilution de N. En outre, une faible exportation de N en fin de culture a été observée (i.e., en moyenne 60 kg de N ha^{-1}). La plante a également montré quelques traits de conservation des ressources avec une densité plus élevée de tissus racinaires et un investissement préférentiel vers la construction de ses racines en phase d'établissement. Ce dernier trait peut expliquer un taux de croissance relativement plus faible par rapport aux céréales annuelles comme le blé. Finalement, sa forme cespiteuse, produisant des ramets rapprochés, induit une propagation lente et radiale de la plante. Cette stratégie de développement pourrait lui conférer une bonne tolérance aux conditions stressantes et une utilisation des ressources locales efficiente (i.e., stratégie de monopolisation). Cependant, sa phase lente d'établissement nécessite de trouver de nouvelles solutions pour limiter la concurrence des plantes désirées ou non au sein de la parcelle.

Un certain compromis a été observé entre la croissance végétative, impliquant un investissement suffisant dans les organes permanents, et la production de grains. En effet, les excès de biomasse et de densité de talles en début de saison ont impacté né-

gativement le rendement par épi. Bien que cette production de grain soit actuellement faible (i.e., moyenne d'1 t ha^{-1}), un résultat majeur est qu'elle est restée constante sur quatre années de culture, ce qui n'avait jamais été observé dans d'autres études. Sous nos conditions pédoclimatiques (relativement favorables au développement de la culture), ce rendement a été expliqué par une fertilité des talles et des épis stable. Le moment optimal pour l'initiation des talles reste à déterminer, mais celui-ci doit être précoce. Un semis relativement dense à la fin de l'été a permis de maximiser la fertilité des talles. Lors des années de repousse, la surproduction de talles et d'épis devrait être évitée par un broyage des résidus de récolte au niveau du sol et une défoliation à l'automne. Ce qui peut également prévenir l'obstruction de la lumière au niveau de la base des plantes. Finalement, une fertilisation de 50 kg de N ha^{-1} à l'automne combinée à une fertilisation de 50 kg de N ha^{-1} en début de printemps ont été intéressantes pour supporter la fertilité des talles tout en limitant le risque de biomasse excessive.

L'espèce n'est pas adaptée à une gestion fourragère intensive. En effet, l'initiation de la repousse a été très faible lorsque les méristèmes apicaux ont été coupés lors de l'élongation des tiges. La gestion fourragère semble donc uniquement pertinente dans un contexte à double valorisation grain-fourrage. Les fauches de printemps et d'automne ont été caractérisées par des rendements fourragers faibles (i.e., maximum de 2 t de MS ha^{-1}) mais avec une bonne qualité fourragère proche de celle d'une herbe fraîche. La récolte des résidus de culture à maturité des grains a été caractérisée par de bons rendements fourragers (i.e., de 5 à 15 t de MS ha^{-1}) mais avec une qualité fourragère faible, proche de celle de pailles. Dans le contexte de double valorisation, la fauche de printemps devrait être évitée car elle a réduit le rendement en grain tandis que la repousse observée à l'automne était généralement trop faible pour justifier une fauche. L'association à une légumineuse fourragère pourrait donc être intéressante afin d'augmenter les productions lors de la période automnale.

L'intégration de céréales pérennes au sein des agroécosystèmes nécessite donc de repenser leurs stratégies de gestion tandis que la sélection génétique demeure cruciale pour y parvenir. Celle-ci devrait aider à la création de nouveaux cultivars adaptés aux différents contextes de productions des agriculteurs intéressés par l'implantation de ce type de culture novatrice.

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1

Introduction

L'être humain n'a de cesse de façonner son environnement et de contrôler le cycle biologique des espèces domestiquées afin de lui procurer un approvisionnement permanent en ressources nourricières et non alimentaires utiles à la société. Pour ce faire, les biomes ont progressivement été modifiés avec une réduction des surfaces semi-naturelles au profit d'environnement anthropisés comme les prairies, les terres arables ou encore les zones urbaines ou industrielles (Ellis et al., 2020). Actuellement, les systèmes agricoles couvrent plus de 40 % des terres mondiales habitables (Ritchie et al., 2022) et sont assez intensifs en ressources. En effet, l'agriculture a connu de nombreuses transformations via les différentes révolutions tant agricoles qu'industrielles ou via l'intensification du commerce mondial. Alors que l'expansion des terres agricoles a ralenti à la fin du 20ème siècle, les rendements en termes de nourriture produite par unité de surface de terres ont considérablement augmenté afin de soutenir les besoins alimentaires de la population mondiale croissante (Matson et al., 1997). Cette gestion intensive des terres agricoles, permise par les avancées scientifiques et technologiques, a conduit à l'utilisation de variétés à hauts rendements, d'engrais de synthèse, de produits de protection de plante ou encore d'une mécanisation importante. Dans les pays développés, cela fait référence à la 'révolution verte' qui a débuté dans les années 1960. Cependant, cela a soulevé quelques préoccupations en termes de durabilité à long-terme de ces systèmes et de conséquences environnementales. En effet, l'intensification agricole peut entraîner des conséquences négatives locales comme la réduction de la fertilité du sol ou de la biodiversité, régionales comme la pollution des eaux souterraines ou l'eutrophisation des eaux de surface ainsi que globales comme des impacts sur les constituants atmosphériques et le climat (Matson et al., 1997). Si l'on prend l'exemple des émissions de gaz à effet de serre, la production alimentaire représente 26 % des émissions mondiales dont 27 % (i.e., 7 % des émissions mondiales) proviennent de la production végétale (i.e., l'oxyde nitreux (N_2O) provenant de l'application des engrais azotés organiques ou minéraux ou le dioxyde de carbone (CO_2) provenant des machines agricoles) (Ritchie et al., 2022). Cependant, le système alimentaire mondial repose sur des millions de producteurs différents (e.g., la taille moyenne des exploitations varie de 0,5 hectare au Bangladesh à 3 000 hectares en Australie, ou l'utilisation moyenne d'engrais minéraux varie de 1 kg d'azote par hectare en Ouganda à 300 kg en Chine). L'impact environnemental de cette production alimentaire peut donc varier d'un facteur 50 entre les différents producteurs d'un même produit, ce qui crée des possibilités considérables pour atténuer les impacts environnementaux (Poore and Nemecek, 2018).

Pour atteindre les objectifs environnementaux en Europe occidentale, une base juridique pour les politiques environnementales européennes affectant les pratiques agricoles a émergé durant les dernières décennies. Des législations environnementales telles que la directive nitrates (1991) ou la directive de l'utilisation durable des pesticides (2009) donnent un cadre global pour la protection de l'eau, du sol et de la santé humaine en prévenant les transferts d'intrants synthétiques provenant de l'agriculture. En parallèle, les objectifs environnementaux ont été de plus en plus intégrés au sein de la Politique Agricole Commune (PAC) de l'Union Européenne. Un ensemble de normes appelées 'bonnes conditions agricoles et environnementales' doivent être respectées par les agriculteurs européens qui reçoivent des subventions. Ces normes visent à assurer une agriculture durable en ce qui concerne l'érosion des sols, la matière organique des sols, la structure des sols, la biodiversité ou l'eau, grâce à la promotion d'un ensemble de pratiques agro-environnementales bénéfiques, telles que la couverture des sols, la rotation des cultures, la protection des pâturages permanents, la protection des zones en pente ou l'implantation de bandes herbeuses tampons (Matthews, 2013). Parallèlement, une expansion des pratiques biologiques ou de conservation dans les systèmes agricoles européens est observée, ce qui met en évidence la volonté des agriculteurs de trouver des pratiques innovantes (Duchene et al., 2019). En 2010, l'agriculture de conservation représentait 26 % des terres arables en Europe (Kertesz and Madarasz, 2014) tandis que l'agriculture biologique représentait, en 2020, 9 % de l'ensemble des terres agricoles de l'union européenne (European Commission, 2023).

Comme le souligne da Silveira Pontes (2006), l'intégration de la limitation des dommages environnementaux et la sécurité alimentaire comme éléments essentiels de la performance des systèmes agricoles déplace leurs défis vers une agriculture plus multifonctionnelle. L'objectif est donc de concilier la productivité et la qualité des produits avec la préservation de l'environnement et de la biodiversité tout en assurant la viabilité socio-économique des systèmes. Pour limiter le réchauffement climatique, la décarbonisation du système énergétique mondial est essentielle, mais la transition vers des systèmes alimentaires agissant plutôt comme des puits que comme des sources d'émissions de carbone et protégeant les puits de carbone dans les écosystèmes naturels est tout aussi importante (Willett et al., 2019). Ainsi, l'intensification écologique des systèmes agricoles semble essentielle pour réduire les besoins en ressources externes afin de produire de manière plus durable en augmentant le recours aux fonctions écosystémiques. A l'échelle des productions végétales, le semis direct, l'utilisation d'espèces pérennes ou de légumineuses, les rotations diversifiées des cultures, les cultures de couverture ou la réduction des taux de fertilisation azotée peuvent contribuer à atténuer les émissions de gaz à effet de serre (Sainju, 2016). Toutefois, les résultats environnementaux de beaucoup de ces pratiques sont très variables. Par exemple, le potentiel de stockage du carbone dans le sol varie considérablement en fonction des propriétés du sol, de la pente ou des pratiques antérieures. Ces pratiques ne représentent donc pas une solution environnementale en elles-mêmes mais une option pour les producteurs afin d'atteindre certains objectifs environnementaux (Poore and Nemecek, 2018).

Actuellement, le système alimentaire mondial dépend de plantes annuelles - céréalières, oléagineuses et protéagineuses - couvrant près de 70 % des terres cultivées (Monfreda et al., 2008), ce qui représente environ 50 % de l'apport calorique mondial de l'homme (Kearney, 2010) et constitue d'importantes sources de fourrage pour l'industrie de l'élevage. Cependant, comme l'expliquent les travaux d'Odum (1969), ces objectifs de production agricole entrent souvent en conflit avec la stratégie de 'protection maximale' des écosystèmes. En effet, la stratégie de développement des écosystèmes, ou succession écologique, est l'évolution prévisible des communautés au sein de leur environnement physique. Cette succession résulte en un écosystème stabilisé où la biomasse maximale et les fonctions symbiotiques entre les organismes sont maintenues par le flux d'énergie disponible dans cet écosystème qui bénéficie d'une 'protection maximale' par rapport aux perturbations externes. La culture de plantes annuelles nécessite de faire régresser l'écosystème à un état antérieur et de le maintenir dans cet état année après année, ce qui est coûteux en termes d'énergie. En effet, aux premiers stades de développement de l'écosystème, la production brute est élevée mais n'est pas soutenue par le flux d'énergie de l'écosystème. Il est également caractérisé par des espèces spécialisées à croissance rapide, la conservation des nutriments est médiocre avec des cycles minéraux ouverts et des symbioses internes très peu développées (Odum, 1969).

Les progrès récents en matière de domestication et de sélection des cultures céréalières pérennes offrent une possibilité de réintroduire des polycultures pérennes au sein des agroécosystèmes et d'y régénérer les fonctions des écosystèmes naturels (Glover et al., 2010; Pimentel et al., 2012; Ryan et al., 2018). Les cultures pérennes ont des structures racinaires très étendues et une couverture végétale permanente ce qui peut améliorer les cycles des matières comme l'eau ou les nutriments (Culman et al., 2013; Pimentel et al., 2012), réduire l'érosion des sols (Pimentel et al., 2012; Ryan et al., 2018), réduire les contaminations en intrant synthétiques (Jungers et al., 2019), améliorer la fertilité des sols ou encore renforcer la biodiversité (Culman et al., 2010; Helms et al., 2020). *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey est une graminée en C3 actuellement proposée comme céréale pérenne à double usage, produisant à la fois du fourrage et des grains destinés à l'alimentation humaine. Ses grains pourraient remplacer ceux du blé annuel dans diverses applications (e.g., en grains entiers, produits de boulangerie ou en entrant dans la composition de bières ou de whisky) et bénéficient d'investissements et de transformation agroalimentaires de la part d'industries américaines telles que General Mills ou de Pantagonia Provisions (Duchene et al., 2019). En 2021, elle était cultivée sur environ 1600 hectares en Amérique du Nord, contre 200 hectares en 2019, en agriculture conventionnelle ou biologique régénérative certifiée ou en transition (Peters, 2022). Ce type de culture céréalière pérenne pourrait donc répondre aux objectifs environnementaux de l'union européenne et satisfaire aux besoins de diversification de certains agriculteurs. Toutefois, les cultures céréalières pérennes en développement seront traitées comme de nouveaux produits alimentaires, ce qui nécessitera le développement de nouvelles chaînes d’approvisionnement. Le succès commercial d’une nouvelle culture devrait nécessiter la coordination de multiples facteurs, notamment la qualité des semences, les méthodes de lutte contre les différents bioagresseurs, l’équipement de terrain approprié, l’accès aux installations de transformation avec une expertise à chacune des étapes, de la manipulation des semences à la récolte ou à la formulation de recettes (DeHaan et al., 2023).

2

Etat de l’art

1. Développement d'espèces pérennes à intégrer dans les systèmes agricoles

Comme décrit par Wagoner (1990), l'utilisation de grains récoltés d'espèces pérennes n'est pas un concept nouveau avec de nombreux exemples tout au long de l'histoire de l'humanité. *Leymus arenarius* (L.) Hochst., le seigle des mers, ou 'wildrye' en anglais, était par exemple cultivés par les colons vikings d'Islande, du Groenland et de Terre-neuve tandis que les indiens Cucapa récoltaient les graines de *Distichlis palmeri* (Vasey) Fassett ex I.M.Johnst., le nipa, pour en faire du pain et de l'atole dans le nord-ouest du Mexique. Actuellement, des programmes de recherche visant à identifier et à améliorer les cultures pérennes ont été lancés en Argentine, en Australie, en Chine, en Inde, en Suède et aux États-Unis et sont désormais étendus au monde entier (Glover et al., 2010). En effet, il existe toute une série de cultures pérennes à divers stades de développement et adaptées à divers environnements et utilisations (DeHaan et al., 2023). Des espèces pérennes et des populations de plantes hybrides dérivées de parents annuels et pérennes, destinées à être utilisées comme cultures céréalières, oléagineuses ou protéagineuses, sont testées : riz, blé, seigle, orge, sorgho, lin, pois d'Angole, sainfoin ou silphie (Glover et al., 2010; The Land Institute, 2024a). La faisabilité économique de ce type de culture découle de la réalisation d'objectifs de rendements. Les techniques culturales sont essentielles pour le développement de ces modèles de culture mais ne seront probablement pas suffisantes pour atteindre ces objectifs. Comme pour les céréales annuelles, les efforts de sélection végétale seront certainement à l'origine d'une part significative de l'augmentation des rendements de ces espèces pérennes (Wagoner, 1990).

Deux approches différentes sont utilisées pour développer ces cultures pérennes : la pérennisation des espèces annuelles via une large hybridation avec des plantes pérennes apparentées ou la domestication de plantes pérennes sauvages. En théorie, les larges hybridations rendent disponibles les gènes contrôlant les caractéristiques nécessaires à la production de grains (e.g., taille des grains ou capacité de battage) et les gènes de pérennité. Bien que ces espèces annuelles et pérennes sauvages soient apparentées, certaines difficultés génétiques liées à des différences de niveaux de ploïdie, à des incompatibilités génomiques, à des déséquilibres cytoplasmiques ou à des problèmes de stérilité sont observées. Au-delà des problèmes de stérilité et de compatibilité croisée, le transfert des caractères des espèces sauvages apparentées peut s'avérer difficile (Wagoner, 1990). Des croisements ont effectivement pu être réalisés entre des espèces annuelles, telles que le blé, le seigle, le maïs et le sorgho, et leurs parents pérennes respectifs. Cependant, le transfert des caractéristiques pérennes souhaitées est peu fructueux. Les hybrides résultant de ces croisements ont tendance à exprimer plus fortement le comportement annuel que pérenne tandis que les isolats pérennes ont tendance à présenter des caractéristiques agronomiques peu désirables

(Hayes et al., 2018; Wagoner, 1990). La domestication directe des plantes pérennes sauvages présente l’avantage de disposer de populations de plantes pérennes vigoureuses, tandis que toutes les autres caractéristiques facilitant la récolte et l’utilisation des grains doivent être développées. La sélection des espèces pérennes comme nouveau modèle de culture dépendra, dans le futur, de nombreux facteurs. Leur potentiel de production de grains dans l’environnement où elles seront cultivées devra être évalué (e.g., capacité de battage, maturité synchrone des graines, taille correcte des grains (>2,0 mg) ou épis maintenus au-dessus du niveau du feuillage). En outre, d’autres facteurs nécessaires à la facilitation de leur développement devront être pris en compte (e.g., variabilité suffisante au sein de l’espèce ou stabilité méiotique) (Wagoner, 1990).

Comme décrit par Wagoner (1990), les efforts visant à pérenniser certaines espèces annuelles (i.e., blé, seigle, maïs ou sorgho) par le biais d’une large hybridation ont été initiés au cours du 20ème siècle à différents moments dans le monde, avec les investissements les plus importants consacrés au développement du blé pérenne. En 1930, le premier croisement fructueux entre des espèces annuelles et pérennes de Triticeae a été réalisé en Union soviétique. Diverses espèces de blé ont été croisées avec la graminée fourragère pérenne *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey (i.e., l’agropyre intermédiaire ou ‘intermediate wheatgrass’ en anglais). Parallèlement, des tentatives de développement de seigle pérenne ont également été effectuées en hybridant le seigle annuel (*Secale cereale* L.) avec son parent pérenne (*Secale montanum* Guss.). Les efforts se sont ensuite étendus à d’autres régions et des tentatives ont été réalisées pour développer des céréales véritablement pérennes. Ces programmes ont généralement produit des plantes pérennes à courte durée de vie avec des rendements inférieurs à ceux des céréales annuelles et sans débouchés commerciaux clairs. Cette approche, éprouvée par intermittence depuis près d’un siècle, est toujours d’actualité car ces hybrides nécessitent des solutions innovantes pour les rendre viables (DeHaan et al., 2023). Le riz pérenne fournit actuellement un exemple de ce qui peut être réalisé grâce à un investissement soutenu dans un programme de sélection. Un premier croisement entre le riz annuel (*Oryza sativa* L.) et son parent pérenne (*Oryza longistaminata* A.Chev.& Roehr.) a été obtenu en 1996 et, en 2007, la première descendance prometteuse F2 a ensuite été identifiée avec une bonne production de semences et une production modérée de rhizomes. Ensuite, des générations d’autofécondation et de sélection de plantes présentant une viabilité pollinique accrue et des rhizomes courts, ainsi que des cycles ultérieurs de rétrocroisements avec divers types de riz élites ont été réalisés. En 2018, la première variété de riz pérenne, PR23, a été mise à la disposition des agriculteurs chinois, avec une forte capacité de repousse et un rendement soutenu (i.e., 7 t ha^{-1} par récolte, similaire au rendement du riz annuel replanté). Ce riz pérenne a permis de réaliser des économies substantielles en termes de main-d’œuvre et d’intrants, ce qui a dynamisé les revenus nets des agriculteurs tout en offrant les avantages environnementaux attenus. La réduction des perturbations du sol a par exemple entraîné des améliorations mesurables au niveau du sol, avec une augmentation de 0.95 t ha^{-1} de la teneur en carbone organique suite à quatre ans de production (DeHaan et al., 2023; Zhang et al., 2023). Cependant, ce succès doit être mesuré car le programme de sélection du riz pérenne bénéficie d'un avantage fondamental par la possibilité de recombinaison entre les chromosomes correspondants provenant du riz annuel et pérenne. Comme expliqué par DeHaan et al. (2023), chez le blé, les parents annuels et pérennes ont divergé au point que les chromosomes ne s'apparient pas facilement, d'où la nécessité de disposer de nouvelles techniques moléculaires pour développer le blé pérenne. À présent, le génome de référence du parent pérenne actuellement utilisé dans les hybrides de blé pérenne ainsi que les méthodes de séquençage, d'édition du génome ou de mutagénèse sont disponibles, et leurs applications intensives pourraient conduire à des avancées capitales pour le développement du blé pérenne (DeHaan et al., 2023).

La domestication des cultures pérennes est surtout centrée aux Etats-Unis (Wagoner, 1990) avec des acteurs majeurs comme le Land Institute (Salina, Kansas, USA) étendant internationalement leurs collaborations. L'institut développe par exemple la silphie pérenne (*Silphium integrifolium* Michx.) par domestication directe d'une plante indigène d'Amérique du Nord afin d'être aussi productive que le tournesol oléagineux. Cette espèce sauvage possède de relativement grosses graines et un rendement élevé, mais les insectes nuisibles et les maladies présents dans son aire d'origine représentent un défi particulier pour son programme de sélection (DeHaan et al., 2023; The Land Institute, 2024b). Le sainfoin (*Onobrychus vicifolia* Scop.) est une légumineuse fourragère aussi domestiquée par le Land Institute. Elle possède des rendements appréciés dans les régions sèches, cependant ses grains doivent encore être testés pour déterminer s'ils sont sans danger pour la consommation humaine (DeHaan et al., 2023). Le développement de l'orge pérenne est possible tant par large hybridation entre l'orge annuelle (*Hordeum vulgare* L.) et l'orge pérenne (*Hordeum bulbosum* L.), que par domestication directe de l'orge pérenne. Cependant, une faible recombinaison entre les chromosomes annuels et pérennes a été observée dans le cadre d'une large hybridation. La domestication directe de *H. bulbosum* représente désormais une réelle possibilité grâce aux informations génomiques détaillées disponibles pour cette orge ainsi qu'aux méthodes d'édition du génome ou de mutagenèse (Chapman et al., 2022; Westerbergh et al., 2018). Enfin, le dernier et le plus célèbre exemple de domestication directe d'une espèce pérenne est celui de *Thinopyrum intermedium*, actuellement développé comme culture céréalière pérenne. En 1983, près de 100 espèces de graminées pérennes ont été évaluées au Rodale Research Center (Kutztown, Pennsylvania, USA), avant de choisir *Thinopyrum intermedium* (DeHaan et al., 2014) pour ces nombreuses caractéristiques agronomiques nécessaire à sa domestication. Nous pouvons citer les épis sur des tiges relativement fortes et au-dessus du feuillage, la taille des graines, la facilité de battage, la maturité synchrone des graines, la repousse pérenne vigoureuse, ou les graines appétentes pour la consommation humaine ainsi que la similitude nutritionnelle avec le blé annuel (*Triticum aestivum* L.). En effet, utilisée comme graminée fourragère aux États-Unis, la production mécanisée de semences était déjà réalisée pour approvisionner l'industrie des semences fourragères (Wagoner, 1990). L'Institut Rodale a commencé deux cycles de sélection récurrente en 1988 pour améliorer la fertilité et la taille des graines. En utilisant cette première sélection, le Land Institue a initié son propre travail de domestication, où la sélection récurrente basée sur le phénotype a commencé en 2003, pour le rendement par épi, l'augmentation de la masse des graines, la facilité de battage, la réduction de la hauteur et la maturité précoce. Depuis, des programmes de sélection ont également été mis en place dans différentes universités d'Amérique du Nord et de Suède (Bajgain et al., 2022; DeHaan et al., 2014). Actuellement, une variété commerciale de *Thinopyrum intermedium* sous le nom de Kernza® a été déposée par le Land Institute tandis que le premier cultivar mondial de grain de qualité alimentaire, appelé MN-Clearwater (Reg. no. CV-287, PI 692651), a été développé par l'Université du Minnesota, USA (Soto-Gómez and Pérez-Rodríguez, 2022).

2. Potentiel de *Thinopyrum intermedium* en tant que culture céréalière pérenne

2.1. Description générale

2.1.1. Origines

Thinopyrum intermedium (Host) Barkworth & D.R. Dewey est originaire du sud de l'Union soviétique, de l'Asie centrale et de la région méditerranéenne (Wagoner, 1990). Elle a été introduite pour la première fois en Amérique du Nord dans les années 1930 pour le contrôle de l'érosion des sols, la restauration de la végétation et la production de fourrage (Barkworth et al., 2007; Wagoner, 1994). Son avantage est de se développer et de pousser dans des zones sèches avec des sols sablonneux ou pierreux et donc d'augmenter la productivité des terres marginales (Barkworth et al., 2007; Wagoner, 1994; Jensen et al., 2016). Actuellement, *Th. intermedium* est couramment cultivé comme fourrage (i.e., en pâturage ou en prairie de fauche) dans les grandes plaines et dans les régions inter montagneuses de l'ouest des États-Unis (Ogle et al., 2011; USDA, 2014). Plusieurs cultivars bien décrits ont été développés aux États-Unis pour leurs valeurs fourragères ou leurs rendements fourragers (Ogle et al., 2011; Wills et al., 1998). Comme indiqué précédemment, *Thinopyrum intermedium*, et en particulier sa sous-espèce *intermedium*, est à présent développée en tant que culture céréalière pérenne.

2.1.2. Description phylogénétique

La population de *Th. intermedium* est hétérogène. En effet, l'espèce est essentiellement auto-incompatible et allogame, hexaploïde ($2n = 6x = 42$) avec trois sous-génomes et possède un degré élevé d'hétérozygotie (Altendorf et al., 2021; DeHaan et al., 2014; Jensen et al., 2016). Comme l'ont exposé Wang et al. (2015), le génome de *Th. intermedium* est probablement composé de deux génomes ancestraux de l'actuel génome 'Jb' de *Thinopyrum bessarabicum* (Savul. & Rayss) Á. Löve et 'Je' de *Thinopyrum elongatum* (Host) D. R. Dewey, ainsi que d'un génome 'St' présent chez les espèces du genre *Pseudoroegneria*. Selon Jensen et al. (2016), l'agropyre intermédiaire comprend des types glabres et pubescents via deux sous-espèces. La sous-espèce *intermedium*, le type glabre, et la sous-espèce *barbulatum* (Schur) Barkwork & D.R. Dewey, le type pubescent (Barkworth et al., 2007). Contrairement à la sous-espèce *intermedium*, aucune collection de l'USDA NPGS de la sous-espèce *barbulatum* n'est active (i.e., semences disponibles). De plus, la différence entre les deux types n'est pas toujours effectuée, car les semences commerciales contiennent souvent à la fois des types pubescents et glabres (Jensen et al., 2016; Ogle et al., 2011).

2.1.3. Description morphologique

Th. intermedium est une plante en C3, à longue durée de vie, principalement cespiteuse mais produisant des rhizomes courts (Bajgain et al., 2020; Ogle et al., 2011). Les plantes atteignent une hauteur de 1 à 1,5 m (Wills et al., 1998). Son inflorescence en épi est composée de multiples épillets sessiles, un à chaque nœud du rachis. Au sein de chaque épillet se trouve une série de fleurons, chacun comprenant une lemme, une paléole et un caryopse avec des niveaux de fertilité variables (Altendorf et al., 2021). Sa morphologie est illustrée à la Figure 2.1.

2.2. Services écosystémiques

2.2.1. Services de productions en alimentation humaine

Les caractéristiques permettant à une espèce de graminée pérenne d'être bien adaptée à son environnement naturel ne sont généralement pas celles qui facilitent son utilisation comme culture céréalière. Par exemple, dans leur habitat naturel, ces graines sont généralement petites, ne se battent pas facilement, mûrissent de manière non synchronisée, tombent au sol ou l'épi se brise facilement. Les stratégies de survie des graminées pérennes tendent à favoriser la reproduction asexuée (i.e., végétative) plutôt que la reproduction sexuée, avec des rendements en grains généralement plus faibles que leurs parents annuels (Wagoner, 1990). Parmi d'autres graminées pérennes, *Th. intermedium* a pourtant été préférée en raison de son potentiel agronomique initial intéressant pour la production de grains, comme par exemple la maturité synchrone de l'ensemble des grains en 4 à 7 jours. Toutefois, la culture est aux prémices de sa domestication et son potentiel de rendement en grain est encore faible par rapport à ses homologues annuels. Elle atteint un maximum de 10 à 20 % du potentiel des meilleures lignées modernes de blé dans un large gamme de conditions pédoclimatiques (Culman et al., 2013; Larkin et al., 2014; Newell and Hayes, 2017), ce qui peut restreindre la viabilité économique de la culture.

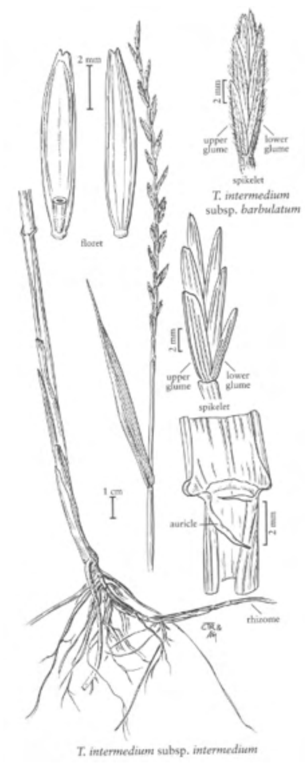

FIGURE 2.1 – Description morphologique de *Thinopyrum intermedium* subsp. *intermedium* © Barkworth, 2007.

Cependant, comme le soulignent DeHaan et al. (2023), la réponse de *Th. intermedium* à la domestication est assez importante avec un potentiel d'amélioration génétique élevé grâce à une variabilité importante au sein de son matériel génétique (Wang et al., 2015) et à une histoire de sélection récente. En effet, une hétérogénéité génomique est toujours présente au sein de cette population de *Th. intermedium* non stabilisée. En utilisant la sélection génomique moderne et si les tendances actuelles se poursuivent, la progression du rendement en grain pourrait atteindre 58 kg ha^{-1} an^{-1} (Bajgain et al., 2022).

Toutefois, différents problèmes de production se posent au champ. Dans ce type de condition, la densité des plantes est généralement plus élevée, et un certain compromis entre la densité de talles et leur fertilité au fur et à mesure que le peuplement vieillit a été observé (Jungers et al., 2017; Hunter et al., 2020a). Ainsi, des densités totales dépassant approximativement 1 000 talles m^{-2} pourraient être préjudiciables au rendement en grains. En outre, on observe une tendance marquée de baisse du rendement avec le vieillissement du peuplement, ce qui entraîne une chute de l'indice de récolte pouvant atteindre 50 % (Culman et al., 2023; Duchene et al., 2023). Certaines études ont mis en évidence des dynamiques de production différentes, avec des rendements en grains maximum la première ou la deuxième année de culture pour ensuite chuter les années suivantes (Bajgain et al., 2020; Bergquist et al., 2022; Jungers et al., 2017; Tautges et al., 2018). Plusieurs hypothèses ont été émises pour expliquer cette baisse de rendement. Notamment, une concurrence accrue pour la lumière ou les nutriments avec une densité de tiges plus élevée (Jungers et al., 2017; Tautges et al., 2018), une stratégie de croissance pour tolérer les stress (Law et al., 2020) ou encore des changements dans l'allocation des ressources en faveur des organes pérennes (Vico et al., 2016).

Le grain de *Th. intermedium* est caractérisé par une petite taille qui est associée à une teneur en protéines plus élevée par rapport aux céréales annuelles, ayant proportionnellement plus d'endosperme amylacé dans leurs plus gros grains (Wagoner, 1990). En effet, par rapport au blé annuel, la farine complète de *Th. intermedium* a une teneur en amidon plus faible (i.e., 47 %) et un profil nutritionnel supérieur (i.e., teneur en protéines plus élevée avoisinant 20 %, teneur en fibres alimentaires de 17 à 19 %, teneur en cendres plus élevée avoisinant 3 %, teneur en acides aminés essentiels plus élevée, à l'exception de la lysine, et teneur en caroténoïdes et en antioxydants plus élevée) (Becker et al., 1991; Marti et al., 2015; Rahardjo et al., 2018; Tyl and Ismail, 2019). Aucune quantité significative de facteurs antinutritionnels (i.e., tanins ou inhibiteurs de la trypsine) n'a été mesurée dans les grains de *Th. intermedium*, ce qui est similaire au blé (Becker et al., 1991). Cependant, la farine complète de *Th. intermedium* pose des problèmes pour les produits de boulangerie, qui nécessitent des propriétés de levée de la pâte. Dans l'étude de Rahardjo et al. (2018), où des tests de cuisson de pains ont été réalisés, les gluténines de haut poids moléculaire étaient déficientes et une teneur élevée en fibres a été observée. Ces teneurs contribuent à la faible capacité de formation du réseau de gluten de *Th. intermedium* par rapport aux farines de blé. Les ratios d'amylose et d'amylopectine entre les deux types de farines de blé et de *Th. intermedium* étaient similaires, mais les farines de *Th. intermedium* présentaient une viscosité plus faible pendant le chauffage et le refroidissement. La stabilité, la résistance à l'extension et l'extensibilité de la pâte de *Th. intermedium* étaient également plus faibles (Rahardjo et al., 2018). Des tests de mouture ont également été effectués pour déterminer les taux de récupération de l'endosperme, qui étaient inférieurs à ceux du blé, de l'ordre de 40 à 50 %, et la fraction de son était plus élevée, en raison de la taille plus petite des graines (Wagoner, 1990).

Concernant la valorisation de ces grains en alimentation humaine, les farines de *Th. intermedium* pourraient convenir aux produits qui ne nécessitent pas de propriétés de rétention de gaz (e.g., biscuits, tortillas ou crackers) ou aux produits à base de pâte non pétrie qui ne dépendent pas entièrement du gluten (e.g., crêpes ou gâteaux). En outre, des tests de cuisson ont montré que les qualités gustatives des grains de *Th. intermedium* sont très bonnes, avec un goût de noisette. Ces grains peuvent également être cuits en grain entier, en mélange avec du riz (Becker et al., 1991). Enfin, dans l'étude de Marcus and Fox (2022), le potentiel pour les industries du malt et de la bière a été évalué. Ils ont indiqué que *Th. intermedium* pourrait être un candidat intéressant pour la production de malt et de moût avec des techniques spécifiques pour utiliser ce malt le plus efficacement possible. Par ailleurs, les stratégies de transformation, telles que les conditionneurs de pâte, le raffinage de la farine ou le mélange avec de la farine de blé, peuvent être utilisées pour compenser certains des défauts des grains de *Th. intermedium* et ainsi élargir sa gamme de produits (Bharathi et al., 2022). Comme l'a souligné Wagoner (1990), la variabilité génétique au sein de *Th. intermedium* induit également des différences entre les propriétés des farines. Ceci indique qu'il peut y avoir une variabilité suffisante au sein de l'espèce pour sélectionner et développer des lignées pour différents usages culinaires, tout comme il existe différents types de blé pour les pains, les pâtes et les pâtisseries (Wagoner, 1990). En outre, la composition nutritionnelle des grains de *Th. intermedium* change au cours des cycles de sélection successifs. Dans la revue de Bharathi et al. (2022), l'augmentation de la taille des graines a entraîné une diminution de la teneur en protéines et en fibres alimentaires et une augmentation de la teneur en amidon, ce qui peut affecter les propriétés d'utilisation du grain.

2.2.2. Services de productions en alimentation animale

Comme mentionné précédemment, *Th. intermedium* est couramment cultivée pour le fourrage dans les régions des Grandes Plaines et intermontagneuses de l'Ouest, avec différents cultivars disponibles (Ogle et al., 2011). D'anciennes études ont suggéré que *Th. intermedium* pouvait être utilisée pour la fenaison (Lawrence and Ashford, 1966) ou le pâturage de printemps et d'automne (Moore et al., 1995). En effet, l'espèce peut représenter une source de fourrage intéressante pour les systèmes agricoles de zones sèches, comme dans les Grandes Plaines d'Amérique du Nord, la région des prairies canadiennes ou en Nouvelle-Zélande (Heinrichs and Clark, 1961; Moore et al., 1995; Wills et al., 1998). Dans le cadre d'une utilisation moins intensive, *Th. intermedium* a une capacité de rendement en foin supérieure à celle d'autres graminées (Heinrichs and Clark, 1961). Cependant, en cas de défoliation fréquente et répétée, dans le cadre d'un pâturage continu, la vigueur du peuplement de la graminée peut être diminuée, ce qui suggère que *Th. intermedium* n'est pas adaptée à un pâturage de longue durée (Heinrichs and Clark, 1961; Campbell, 1961).

Grâce à la sélection pour le rendement en grains, *Thinopyrum intermedium* est actuellement proposée comme une culture à double usage, en produisant à la fois du fourrage et des grains (Hunter et al., 2020b). Comme le suggèrent Culman et al. (2023), la majeure partie de sa productivité primaire nette est dirigée vers des structures non reproductives. Les stratégies à double usage, valorisant le fourrage en plus du grain, pourraient donc représenter une potentielle voie viable pour accroître la productivité et donc la rentabilité de la culture. Ces stratégies de double usage pourraient soit être mises en œuvre au cours d'une année culturale, soit entre deux années culturales comme illustré sur la Figure 2.2. Dans la perspective de l'exploitation des grains et du fourrage au cours d'une année de culture, les grains et la paille sont récoltés en été et du pâturage ou des coupes supplémentaires peuvent être effectués à l'automne, au début du printemps ou les deux pour la production de fourrage (Hunter et al., 2020b; Culman et al., 2023). Les récoltes supplémentaires de fourrage au printemps, à l'automne ou les deux augmentent généralement la production totale de biomasse de *Th. intermedium* par rapport à une récolte unique lors de la récolte des grains en été (Hunter et al., 2020b). Dans l'étude de Culman et al. (2023), la récolte de fourrage au printemps a donné la biomasse fourragère la plus faible, tandis que la récolte d'été a donné la biomasse la plus élevée, la récolte d'automne a été intermédiaire. Bien que la qualité du fourrage de la récolte d'été (i.e., la paille) soit relativement faible, Hunter et al. (2020b) ont souligné que la quantité importante de biomasse est particulièrement intéressante car elle fournit un deuxième débouché en plus des grains. Dans un contexte où le rendement en grains diminue lorsque le peuplement de *Th. intermedium* vieillit, une perspective serait de passer d'une gestion de la culture pour la production de grains vers une gestion unique pour la production fourragère (Figure 2.2 ; Culman et al. (2023)). Cela serait possible par la stabilité, au fil des années, de la quantité et de la qualité du fourrage de *Th. intermedium*.

A l'instar des autres graminées fourragères, la qualité fourragère de *Th. intermedium* dépend de son stade phénologique au moment de la récolte. Au début du printemps, avant ou au début de la croissance reproductive, la proportion de feuilles est nettement supérieure à celle des tiges et représente plus de 80 % de la biomasse aérienne (Barriball, 2020). Après ce stade, la proportion de tiges augmente jusqu'à l'émergence complète des épis. Ce changement progressif au cours de la saison de croissance est lié à une augmentation de la biomasse aérienne totale, mais il entraîne une baisse significative de la qualité du fourrage (Thomas et al., 2021; Wróbel et al., 2023). Dans l'étude de Culman et al. (2023), la récolte de fourrage au printemps a donné la meilleure qualité de fourrage tandis que la récolte d'été a donné la moins bonne qualité fourragère, la récolte d'automne était intermédiaire. Comme mentionné dans l'étude de Favre et al. (2019), le fourrage *Th. intermedium* convient aux vaches viandeuses allaitantes, aux vaches laitières et aux génisses lorsqu'il est récolté au printemps et à l'automne, tandis que les résidus de culture, représentés par la paille produite en été, pourraient remplacer la paille dans les régimes nutritionnels laitiers à haute teneur en amidon pour maintenir un bon fonctionnement du rumen.

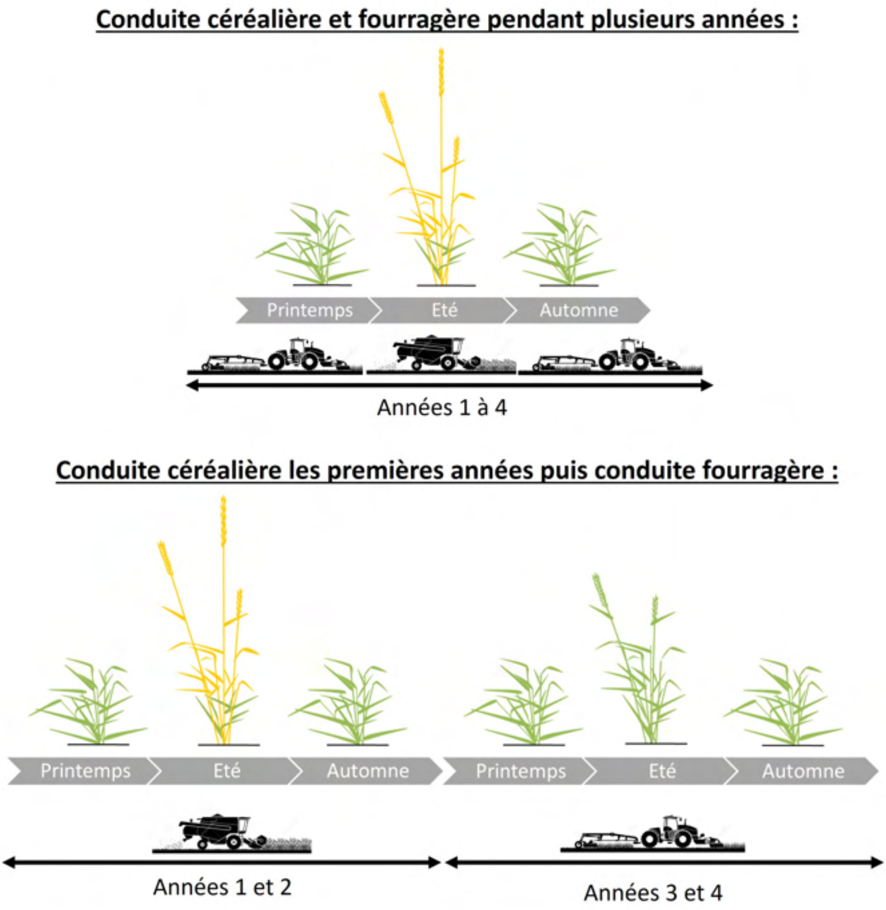

FIGURE 2.2 – Illustration de conduites à double usage de la culture de *Th. intermedium*.

2.2.3. Services de support et de régulation environnementaux

La multifonctionnalité de *Th. intermedium* comme illustrée à la Figure 2.3, réside dans la réalisation de divers services écosystémiques de production mais également de support et de régulation grâce à sa couverture permanente du sol et à son système racinaire persistant (Crews et al., 2016; Rasche et al., 2017; Ryan et al., 2018). En effet, la culture alloue beaucoup de biomasse dans son système racinaire, dans l'étude de Sprunger et al. (2018a), 23 à 50 % de sa biomasse totale étaient alloués à ses racines, contre seulement 10 % pour le blé. La biomasse de ses racines a été estimée être de trois à douze fois supérieure à celle du blé (Sprunger et al., 2018a). Cela conduit, au champ, à une colonisation par ses racines dans les 60 premiers centimètres du sol de 90 % contre 60 % pour les céréales annuelles (Duchene et al., 2020).

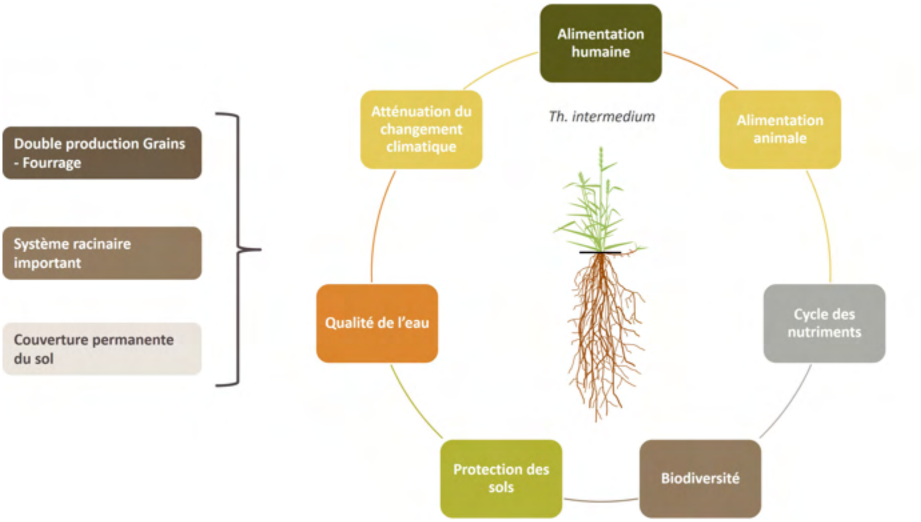

FIGURE 2.3 – Illustration des trois grandes caractéristiques de *Th. intermedium* menant à sa multifonctionnalité.

Sous les cultures pérennes, une contribution à la régulation du changement climatique peut être attendue par une augmentation du stockage du carbone dans le sol. Ce stockage de carbone devrait être lié à deux mécanismes : la réduction des perturbations régulières du sol associées à l'implantation des cultures annuelles, qui exacerbent les pertes par minéralisation (Crews et al., 2016) et l'augmentation simultanée des apports de carbone grâce à une potentielle augmentation de production de biomasse et à une allocation souterraine plus importante (Sprunger et al., 2019). de Oliveira et al. (2018) ont estimé que *Th. intermedium*, sur cinq ans de culture, pouvait représenter un puit de carbone conséquent en stockant en moyenne 370 g C m^{-2} par an, ce qui est, dans leurs conditions, considérablement plus élevé que dans les systèmes de culture annuelle. Toutefois, ce stockage de carbone est variable et dépend de nombreux facteurs tels que les conditions pédoclimatiques (e.g., humidité du sol qui pourrait réduire le stockage de carbone) ou la quantité de biomasse récoltée, qui est également influencée par différents facteurs (e.g., gestion au champ) (de Oliveira et al., 2018). En outre, il a été observé que *Th. intermedium* présentait des rapports C :N des racines principales, supérieurs à ceux du blé annuel, ce qui démontre une plus grande capacité à stocker le carbone dans ses racines par rapport aux systèmes annuels (Sprunger et al., 2019). Dans l'étude de Sprunger et al. (2018b), il a été estimé que *Th. intermedium* devrait être cultivé pendant au moins quatre ans pour observer des avantages en matière de piégeage du carbone par rapport aux cultures céréalières annuelles.

Les plus grandes quantités de carbone du système racinaire (i.e., biomasse racinaire et rapports C :N plus élevés) et les réservoirs de carbone stabilisés du sol ont induit un changement dans la structure et le fonctionnement du réseau trophique sous culture de *Th. intermedium* par rapport aux systèmes annuels (Sprunger et al., 2019). Dans l'ensemble, pour une culture de *Th. intermedium* pleinement établie (i.e., âgée d'au moins trois ans), des réseaux trophiques hautement structurés et plus complexes que le blé annuel ont été favorisés. L'étude au champ de Förster et al. (2023) a révélé que les vers-de-terre sont avantagés par les systèmes de culture pérennes, avec la promotion d'espèces épigées et endogées, d'une communauté plus riche en espèces et un taux de reproduction plus élevé des vers-de-terre par rapport à des sols sous culture de blé annuel. Ces communautés de vers-de-terre plus diversifiées et plus abondantes pourraient s'expliquer par l'amélioration de leur habitat grâce à la réduction du travail du sol, la croissance plus importante des racines ou par l'augmentation de la source de nourriture par rapport au blé annuel (Förster et al., 2023). Enfin, une favorisation de la biomasse fongique du sol et divers indicateurs de champignons mycorhiziens à arbuscules ont également été observés sous *Th. intermedium* par rapport aux cultures annuelles (Duchene et al., 2020). En outre, la couverture permanente du sol tout au long de l'année est également bénéfique pour l'accroissement de la biodiversité en offrant un habitat pour la faune tels que les oiseaux ou les mammifères sauvages.

Plusieurs études ont mis en évidence une plus grande efficacité de *Th. intermedium* à utiliser les ressources du sol et donc à soutenir le cycle des nutriments. En effet, l'espèce se caractérise par un système racinaire profond et étendu, en plus de sa période de croissance prolongée, son exploration du profil du sol est améliorée dans l'espace et dans le temps (Culman et al., 2013; Duchene et al., 2020; Jungers et al., 2019). Comme le montre l'étude de Sprunger et al. (2018a), l'efficacité d'utilisation de l'azote (i.e., teneur en azote de la plante entière (racines comprises) divisée par l'azote disponible) de la culture pérenne est assez élevée. La plante semble pouvoir assimiler de grandes quantités d'azote, même supérieures à celles qui ont été appliquées. L'amélioration de la capture de l'azote, tout en réduisant la lixiviation des nitrates (Jungers et al., 2019), suggère que les sources externes d'azote pourraient être minimisées sans entraver les productions de la culture, avec l'avantage de limiter les coûts économiques et environnementaux. *Th. intermedium* possède également une bonne capacité à utiliser l'eau des couches profondes du sol et à maintenir une haute efficacité d'utilisation de l'eau (i.e., le rapport entre l'assimilation ou la productivité du carbone et la perte d'eau) tout au long de la saison de croissance (Clément et al., 2022; de Oliveira et al., 2018). L'amélioration de la capture de l'eau, permettant une certaine tolérance à la sécheresse, confère à la culture un avantage pour une plus grande résilience face au changement climatique (Clément et al., 2022).

La couverture permanente du sol et la structure racinaire extensive des plantes pérennes entraînent généralement une meilleure infiltration de l'eau et une réduction de l'érosion éolienne et hydrique du sol (Pimentel et al., 2012; Ryan et al., 2018). En outre, l'accumulation de matière organique provenant de l'augmentation de la biomasse racinaire par rapport aux cultures annuelles, devrait également favoriser la structure du sol et la stabilité des agrégats ainsi que l'infiltration de l'eau (Wagoner, 1990). Par conséquent, les ressources en eau de surface devraient être préservées grâce à la réduction du ruissellement sous ce type de culture. *Th. intermedium* possède également un rôle clé dans la prévention de la lixiviation des nutriments, avec une réduction de plus de 90 % de la lixiviation du NO3-N par rapport au maïs, comme l'ont observé Jungers et al. (2019), démontrant une capacité pour protéger la qualité de l'eau en réduisant les contaminations des bassins versants.

Un dernier service de régulation des maladies peut également être mis en évidence. En effet, les espèces du genre *Thinopyrum*, y compris *Thinopyrum intermedium*, sont considérées comme les plus proches parents pérennes du blé tendre (*Triticum aestivum*) (Larson et al., 2019). Ce genre est bien connu et utilisé dans le programme de sélection du blé pour ses gènes de résistance à diverses maladies du blé tendre comme la rouille brune des feuilles (*Puccinia triticina* Eriks.), la rouille noire des tiges (*Puccinia graminis* Pers. :Pers.) l'oïdium (*Blumeria graminis* (DC.) E.O. Speer) ou les virus BYDV ('Barley yellow dwarf virus') et WSMV ('Wheat streak mosaic virus') (Li and Wang, 2009).

La valeur des services environnementaux pour les producteurs, les collectivités ou la société aux États-Unis est actuellement soutenue par la subvention fournie par le 'Conservation Stewardship Program' (NRCS-USDA, 2023). Comme le mentionnent Duchene et al. (2019), les agriculteurs européens pourraient également recevoir des paiements provenant de la PAC de l'union européenne, car les céréales pérennes remplissent de nombreuses exigences actuelles (e.g., couverture des sols, réduction du travail du sol ou protection des zones sensibles). La valeur des services écosystémiques pourrait également résider dans l'accès à de nouveaux marchés à l'avenir, tels que l’eau et les crédits de carbone (Pinto et al., 2022).

3. Ecophysiologie de *Th. intermedium*

3.1. Compromis entre reproduction sexuée et axexuée

Thinopyrum intermedium est une graminée cespiteuse produisant des rhizomes courts (Ogle et al., 2011). La culture peut donc se reproduire asexuellement par le tallage (i.e., production de talles intravaginales) et la croissance rhizomateuse (i.e., production de talles extravaginales) (Bergquist et al., 2022; Lafarge and Durand, 2011) et sexuellement par les grains. L’allocation des ressources aux grains est actuellement faible, avec un indice de récolte d’environ 10 % (Duchene et al., 2023). Alors que l’allocation vers les organes pérennes est importante, la biomasse racinaire correspond à 23 à 50 % de sa biomasse totale (Sprunger et al., 2018a) tandis que les rhizomes représentent 17 % de la biomasse souterraine dans les 10 premiers cm du sol (Sakiroglu et al., 2020). En outre, à la fin de la saison de croissance, une quantité élevée d’azote dans les racines est toujours présente, entre 28 à 55 % de la quantité totale d’azote de *Th. intermedium*, contre seulement 9 à 19 % pour le blé (Sprunger et al., 2018a). Ce modèle d’allocation de l’espèce, sans prioriser la production de grains, bien qu’utile pour sa pérennité et sa tolérance aux stress, pourrait représenter un inconvénient dans son utilisation comme culture céréalière. En outre, la reproduction asexuée pourrait potentiellement détourner les ressources des grains (Vico et al., 2016).

Dans les cultures céréalières annuelles, le schéma de répartition des assimilas change avec la transition du stade végétatif au stade reproducteur. Jusqu’à la floraison, il y a trois principaux puits d’assimilas (i.e., les feuilles initiées, les tiges en développement et les tissus de gaines) avec une accumulation de réserves dans la tige et d’autres tissus, tandis que peu d’assimilas sont dirigés vers le système racinaire. Après la floraison, les dernières feuilles fonctionnelles ainsi que les épis verts agissent comme des sources photosynthétiques pour deux puits concurrents (i.e., les grains et les tiges), mais dans les derniers stades du remplissage des grains, il y a normalement une remobilisation importante des hydrates de carbone du stockage des tiges et des feuilles vers les grains (Hay and Porter, 2006). Parallèlement, pour leur survie à long terme ou dans des conditions difficiles, les plantes pérennes favorisent l’allocation des ressources vers les structures souterraines et de stockage (e.g., racines, rhizomes ou bases des tiges), ce qui leur permet d’absorber et de conserver efficacement les ressources (Roumet et al., 2006; Vico et al., 2016). *Th. intermedium* est caractérisée par une allocation importante de ressources vers son système racinaire (Sprunger et al., 2018a). En général, la production racinaire change en fonction des ressources disponibles pour la plante. Par exemple, en prairie la production de racines cesse juste après la coupe, ou en cours de montaison, pour reprendre en cours de repousse tandis qu’en céréales annuelles cette production racinaire cesse vers l'épiaison, puis régresse ensuite (Lafarge and Durand, 2011). La capacité du système racinaire à être un lieu de stockage pour les nutriments serait liée à la durée de vie des racines représentée par les densités et les diamètres racinaires (Bausenwein et al., 2001). Dans les prairies de graminées, la repousse après une coupe dépend surtout de la mobilisation des assimilas stockés principalement à la base des tiges pour générer la nouvelle canopée (Hay and Porter, 2006) tandis que la remobilisation d'azote depuis les racines apparaît probablement lorsque que les réserves aériennes sont insuffisantes (Bausenwein et al., 2001). Finalement, les rhizomes sont des tiges souterraines capables de produire les systèmes aériens et racinaires d'une nouvelle plante. Pour certaines espèces très rhizomateuses comme Miscanthus giganteus, ces rhizomes représentent le lieu de stockage de nutriments, transférés à partir des parties aériennes à l'automne puis remobilisés vers ces parties aériennes lors de la repousse printanière (Zapater et al., 2017). Dans l'étude de Sakiroglu et al. (2020), il a été montré que des hydrates de carbone non-structurels étaient effectivement stockés dans les rhizomes et les racines de *Th. intermedium* suggérant que ces deux organes sont importants pour la survie et la repousse de l'espèce. La compréhension des mécanismes qui sous-tendent la production de biomasse et l'allocation et l'utilisation de l'azote vers la repousse végétative et la reproduction sexuée au fil du temps est nécessaire afin de développer des recommandations de gestion pour les systèmes de production de *Th. intermedium*.

L'augmentation de la production de grains grâce à la domestication entraîne d'importantes questions quant à l'existence d'un compromis entre l'effort de reproduction et la durée de vie. Certains chercheurs contestent la faisabilité des cultures céréalières pérennes (Cassman and Connor, 2022; Loomis, 2022; Smaje, 2015). Ceux-ci considèrent que les efforts sont trop fastidieux pour surmonter le compromis physiologique entre l'investissement des ressources dans la production de grains pour générer une descendance et la croissance et l'entretien des structures végétatives responsables de la longévité de la plante au travers des années. Tandis que d'autres chercheurs soutiennent qu'il est possible d'utiliser de nouvelles connaissances technologiques et scientifiques (Chapman et al., 2022). Ces structures végétatives, considérées comme compétitives (e.g., système racinaire, grandes tiges), pourraient simplement fournir un plus grand réservoir de ressources à réaffecter aux grains récoltés (DeHaan et al., 2005). Selon DeHaan et al. (2023), la question essentielle qui reste sans réponse est de savoir dans quelle mesure les avantages de la culture pérenne peuvent être conservés lorsque celle-ci est sélectionnée pour augmenter le rendement en grains. Vico et al. (2016) ont mis en évidence une invariance des schémas d'allocation de la biomasse des cultures pérennes subissant la sélection génétique. Celles-ci maintiennent une importante allocation souterraine, ce qui pourrait donc maintenir leur multifonctionnalité. En outre, le succès du riz pérenne montre que la survie des plantes pérennes avec un rendement céréalier élevé devrait être possible (DeHaan et al., 2023).

3.2. Stratégie de conservation des ressources

Comme l'ont souligné Duchene et al. (2021), peu d'attention a été accordée à la compréhension de la régulation écophysiologique du cycle de croissance de *Th. intermedium.* Les traits fonctionnels des plantes peuvent aider à expliquer leurs réponses individuelles aux facteurs biotiques et abiotiques par le biais d'un compromis entre la croissance et la survie et à identifier les synergies et les compromis entre les services marchands et non marchands (Duru et al., 2015; Freschet et al., 2021). Sur la base de travaux théoriques et empiriques antérieurs (voir par exemple Mooney (1972) ou Grime (1974)), les variations dans le fonctionnement des plantes peuvent être liées à un spectre économique allant d'une stratégie d'acquisition des ressources à une stratégie de conservation des ressources. Ces traits d'économie de ressources ont des conséquences sur la performance des plantes dans le continuum de niveau de ressources, induisant un compromis entre un retour sur investissement 'lent' et un retour sur investissement 'rapide' (Reich, 2014). Les espèces végétales situées du côté rapide du spectre de l'économie végétale sont généralement caractérisées par une activité photosynthétique et des taux de respiration plus élevés, une plus grande concentration en nutriments dans les feuilles (e.g., teneur élevée en azote) et une masse foliaire plus faible par unité de surface foliaire (qui est l'inverse de la surface foliaire spécifique). Les espèces végétales situées du côté lent du gradient ont cependant une stratégie opposée et construisent des feuilles avec une plus longue durée de vie, mieux défendues de la prédation avec une concentration en nutriments plus faible et une masse foliaire plus importante par unité de surface (Weigelt et al., 2021; Wright et al., 2004). Comme l'explique la théorie de Tilman (1982), dans des conditions de fertilité faible du sol, le taux d'acquisition des nutriments serait faible et les plantes devraient croître très lentement. Les plantes ayant la capacité d'absorption la plus efficace pour la ressource la plus limitante et/ou la capacité de stocker et de conserver cette ressource grâce à des mécanismes de recyclage interne efficaces seraient plus compétitives. Le concept de 'conservation des ressources' au sein de la plante a donc été mis en évidence : 'lorsque que le temps de résidence d'une ressource dans une plante augmente, cette ressource devient plus efficace et, par conséquent, elle peut être acquise en plus petite quantité pour maintenir la plante en vie' (Lemaire, 2001). Ainsi, les espèces à longue durée de vie des feuilles devraient avoir une demande plus faible en ressources azotées et devraient mieux persister dans un sol pauvre que les espèces à courte durée de vie des feuilles. Dans l'étude de Maire et al. (2009), cette stratégie de conservation de l'azote a été liée à différents traits physiologiques. En effet, certaines espèces de graminées de grandes tailles avec des rendements élevés en azote (i.e., absorption de l'azote par les parties aériennes) et une biomasse racinaire et aérienne élevée peuvent présenter des caractéristiques plus conservatrices telles qu'une efficacité élevée d'utilisation de l'azote par les feuilles combinée à une faible concentration en azote de ces feuilles et à une faible capacité d'absorption par les racines, ce qui est le cas de *Dactylis glome-*

rata ou de *Festuca arundinacea*. Certains traits de conservation des ressources de *Th. intermedium* ont été mis en évidence dans différentes études. Duchene et al. (2021) ont par exemple suggéré que *Th. intermedium* appartiendrait aux plantes à croissance lente et plus 'conservatrices' dans les conditions tempérées françaises et belges grâce à une accumulation plus importante de degrés-jours de croissance jusqu'à la floraison par rapport aux plantes plus précoces (e.g., *Lolium perenne* ou *Festuca pratense*). Ces espèces à floraison tardive sont généralement caractérisées par des peuplements plus hauts, une durée de vie des feuilles plus longue, une surface foliaire spécifique plus faible, une concentration d'azote foliaire et un taux de croissance relatif plus faibles. En outre, une efficacité élevée de l'utilisation de l'azote a été signalée pour *Th. intermedium* (Sprunger et al., 2018a).

En plus des traits foliaires, d'autres traits liés à la production de grains, à la construction des racines secondaires et au développement et au maintien des relations symbiotiques avec les mycorhizes sont importants pour le fitness de la plante (Wright et al., 2004). Contrairement aux traits foliaires, les traits liés à la construction des racines secondaires sont multidimensionnels (Kramer-Walter et al., 2016). En utilisant une base de données globale de traits racinaires, Bergmann et al. (2020) ont identifié deux axes principaux de variation de ces traits entre les espèces végétales. Le premier axe, appelé le gradient de collaboration, représente le compromis entre les espèces ayant des stratégies de 'do-it-yourself' (DIY) et d''externalisation'. Alors que les espèces DIY construisent de longues et fines racines (i.e., faible diamètre racinaire et longueur spécifique de racine élevée) pour explorer le sol et absorber les ressources, les espèces qui externalisent confient l'absorption des ressources aux champignons mycorhiziens à arbuscules 'Arbuscular Mycorrhizal Fungi' (AMF) en construisant des racines plus épaisses avec une fraction de cortex plus importante qui conviennent mieux à l'accueil de ces AMF. Le deuxième axe, appelé le gradient de conservation, représente un compromis entre un retour sur investissement rapide et lent. Alors que les espèces situées du côté rapide du gradient de conservation des racines se caractérisent par une concentration en azote racinaire supérieure à la moyenne, les espèces situées du côté lent du gradient ont une densité de tissus racinaires supérieure à la moyenne (Bergmann et al., 2020; Weigelt et al., 2021). À cet égard, Duchene et al. (2020) ont émis l'hypothèse que certains traits racinaires observés chez *Th. intermedium* pourraient également être liés à une stratégie de conservation des ressources, à savoir la densité plus élevée des tissus racinaires. Enfin, la stratégie de survie à long terme de la culture, avec une allocation réduite des ressources aux grains et des investissements substantiels dans le système racinaire, a également été relevée dans de nombreuses études (Dobbratz et al., 2023; Jungers et al., 2017; Sainju et al., 2017; Sprunger et al., 2018a).

4. Conduite culturale de *Th. intermedium*

Outre la sélection génétique, Bajgain et al. (2022) ont estimé que 31 % de l'augmentation globale du rendement en grains serait liée à une meilleure gestion au champ, comme cela a été le cas pour le blé.

4.1. Etablissement

Un établissement correct des peuplements est essentiel pour la productivité à long terme des cultures pérennes. Les périodes les plus probables pour un établissement réussi de la culture de *Th. intermedium* en production céréalière se situent entre la fin de l'été et le début de l'automne (Jungers et al., 2022). En effet, la culture nécessite une double induction pour l'initiation de sa floraison, avec la vernalisation comme induction primaire et une période de transition vers des jours plus longs et des températures plus élevées comme induction secondaire (Duchene et al., 2021). Les semis de printemps ne permettront donc pas à la culture de produire des grains au cours de la première année. Dans l'étude de Jungers et al. (2022), les rendements en grains et en biomasse étaient les plus élevés lorsque les semis étaient effectués aux dates les plus précoces en fin d'été dans la plupart des environnements, alors que les semis de printemps n'ont produit que peu ou pas de grains au cours de la première année de production. L'augmentation des rendements en grains associée à des dates de semis précoces en automne est probablement liée au développement morphologique plus élevé des plantes avec des talles multiples qui peuvent vernaliser et produire des grains l'année suivante (Jungers et al., 2022).

Comme l'ont observé les études d'Olugbenle et al. (2021) et Pinto et al. (2022), *Th. intermedium* peut souffrir de la concurrence lorsqu'il est semé en même temps qu'une légumineuse dans les les stratégies d'association d'espèces. Le semis de trèfle violet à l'automne en même temps que le *Th. intermedium* a, par exemple, réduit le rendement en grains de la culture par rapport à un semis de trèfle violet semé au printemps, après le semis automnal de *Th. intermedium* (Olugbenle et al., 2021). Pinto et al. (2022) ont suggéré que l'accumulation précoce de biomasse de *Th. intermedium* est essentielle pour que la culture puisse concurrencer l'établissement d'autres espèces plus 'agressives' au sein de la parcelle. En outre, du fait de sa phénologie relativement tardive (Duchene et al., 2021), sa capacité de concurrence envers les adventices est réduite et peut mener à des développements importants d'adventices pendant cette phase d'établissement (Duchene et al., 2023).

Dans la revue de Kruger (nd), diverses études ont testé l'espacement entre les rangs pour la production de grains de *Th. intermedium* en tant que culture fourragère. Globalement, les rendements en grains étaient plus élevés dans les interlignes relativement larges (i.e., de 45 cm à 104 cm), alors qu'ils peuvent diminuer dans les interlignes trop élevées (i.e., de 76 à 152 cm) en fonction des conditions pédoclimatiques. Dans des études plus récentes, des interlignes relativement larges (i.e., de 30 à 61 cm) ont été associées à des rendements en grains plus élevés que des interlignes plus étroites (i.e., 15 cm), mais ne peuvent pas atténuer la baisse de rendement à mesure que le peuplement vieillit (Hunter et al., 2020a). En ce qui concerne les rendements en paille et en fourrage, ils étaient plus élevés dans les interlignes de 15 et 30 cm que dans les interlignes de 61 cm (Hunter et al., 2020b).

4.2. Régulateur de croissance

La productivité de *Th. intermedium* pourrait être limitée par la verse que peut subir la culture et qui est favorisée par l'apport d'azote (Jungers et al., 2017) ou les conditions climatiques. Dans l'étude de Frahm et al. (2018), différents régulateurs de croissance ont été utilisés, comme c'est le cas pour le blé ou d'autres graminées en C3, pour réduire la hauteur des plantes et la verse consécutive des tiges. Le Trinexapacethyl, à la dose habituellement recommandée par les firmes phytopharmaceutiques, a effectivement permis de réduire la hauteur des plantes de 10 à 20 % et la verse jusqu'à 20 % lors d'une année climatique où le risque était élevé, ce qui a entraîné une augmentation du rendement en grains. La production de biomasse n'a pas été influencée par l'utilisation de ce régulateur de croissance, alors que *Th. intermedium* traité avec le Trinexapacethyl avait des tiges plus épaisses par rapport au témoin non traité. La biomasse de la base des tiges a pu être augmentée et a pu ainsi compenser la perte de biomasse due à la réduction de la hauteur. En outre, une hauteur de plantes plus faible grâce à la diminution de la longueur des entre-nœuds est généralement associée à une plus grande allocation photosynthétique vers les grains par rapport à la production végétative, ce qui pourrait conduire à un indice de récolte plus élevé dans la culture (Frahm et al., 2018). Des efforts de sélection de *Thinopyrum intermedium* sont en cours pour augmenter la production de grains en sélectionnant une hauteur de plante réduite afin d'atténuer le risque de verse et d'augmenter les rendements en grains (Frahm, 2017).

4.3. Nutrition

Peu de connaissances sont disponibles sur l'efficience d'utilisation de l'azote par *Th. intermedium*, alors que la gestion de cet élément est un point clé en étant l'un des principaux facteurs limitants des productions agricoles (Gastal et al., 2015). *Th. intermedium* se caractérise par un système racinaire profond et étendu, son exploration du sol et son utilisation des ressources sont meilleures à la fois dans l'espace et dans le temps grâce à une période de croissance prolongée (Culman et al., 2023; Duchene et al., 2020; Jungers et al., 2019). Cette observation suggère que les sources externes d'azote pourraient être minimisées sans entraver les productions de la culture, tout en limitant les coûts économiques et environnementaux. Sprunger et al. (2018a) ont observé que l'efficacité d'utilisation de l'azote par la biomasse aérienne de *Th. inter-*

medium était similaire à celle du blé. Cependant, si l'on tient compte de l'ensemble de la biomasse, tant aérienne que souterraine, cette efficacité d'utilisation était plus élevée pour *Th. intermedium*. Certaines études ont investigué l'impact de la fertilisation azotée sur les performances de la culture (Frahm et al., 2018; Jungers et al., 2017), sans quantifier l'apport d'azote naturel du sol. Jungers et al. (2017) ont constaté que l'optimum se situait entre 61 et 96 kg de N ha^{-1} appliqués au printemps pour maximiser les rendements avec une moyenne de 10,8 t matière sèche (MS) ha^{-1} au stade de maturité des grains. Dans l'étude de Tautges et al. (2018), l'application annuelle de 80 kg de nitrogen (N) ha^{-1} a permis d'atténuer le déclin du rendement en grains avec l'âge du peuplement et a augmenté la biomasse de *Th. intermedium* par rapport au témoin non fertilisé. Néanmoins, des recherches sont nécessaires pour comprendre l'impact de la nutrition azotée sur l'écophysiologie de l'espèce, ce qui entraînera sans aucun doute des conséquences sur les pratiques agronomiques pour répondre aux besoins de la culture.

Concernant les besoins en phosphore et en potassium, ceux-ci ne semblent pas très élevés. Comme le synthétise Kruger (nd), la réponse de *Th. intermedium* est trop faible, en termes de rendements, pour justifier une fertilisation. Seule une correction des carences du sol sera nécessaire au moment du semis, ce qui peut arriver dans des sols de zones arides où des quantités de 50 kg ha^{-1} de P_2O_5 et K_2O seraient utiles.

4.4. Récolte du fourrage

Les opérations de défoliation peuvent avoir un impact majeur sur la productivité des graminées. La défoliation effectuée au-dessus de l'apex est connue pour préserver le potentiel reproductif des talles. Néanmoins, la défoliation peut générer de nouvelles talles végétatives et pourrait éventuellement retarder l'élongation de la tige ou réduire la hauteur de la talle (Gillet, 1973; Mansat and Pfitzenmeyer, 1966; Rouet et al., 2021). En ce qui concerne *Th. intermedium*, diverses options de gestion dans une perspective de double usage ont été étudiées ; soit par fauche (Pugliese et al., 2019; Hunter et al., 2020a,b), soit par pâturage (Dick et al., 2018) et réalisées au début du printemps ou après la récolte des grains en été ou en automne. Divers impacts ont été observés à la fois sur la production totale de biomasse et sur la production de grains par rapport à une récolte unique de grains à maturité de la culture. Hunter et al. (2020b) ont observé que ces récoltes supplémentaires de fourrage augmentaient la production totale de biomasse de la culture. Culman et al. (2023) ont, eux, observé que la récolte de fourrage au printemps et à l'automne augmentait la valeur nutritive totale du fourrage, car ce fourrage a une valeur nutritive plus élevée que celle de la paille récoltée lors de la récolte des grains. L'impact de la récolte du fourrage, en particulier le moment et la fréquence, sur les rendements en grains doit également être pris en considération. La fauche de printemps a tendance à diminuer le rendement en grains (Culman et al., 2023; Zimbric et al., 2021), tandis que la fauche d'été et d'automne peut augmenter le rendement en grains par rapport à aucune récolte de fourrage (Culman et al., 2023; Pugliese et al., 2019), mais sans atténuer la baisse de rendement due au vieillissement du peuplement (Hunter et al., 2020a).

4.5. Association avec des légumineuses

Le mélange de légumineuses et de graminées fourragères est connu pour sécuriser le potentiel de rendement du fourrage (Louarn et al., 2016) et peut améliorer sa qualité. Par rapport à un fourrage de graminées pures, la teneur en protéines peut être renforcée, la teneur en fibres réduite (Baumont et al., 2016) et la digestibilité, et donc la valeur énergétique, améliorée, en fonction de l'espèce de légumineuse et de ses caractéristiques chimiques et morphologiques. De plus, la digestibilité des légumineuses diminue moins rapidement au cours du cycle végétatif que celle des graminées, leur valeur nutritionnelle est donc plus stable dans le temps. Dans l'étude de Favre et al. (2019), le fourrage fourni par le mélange de *Th. intermedium* avec du trèfle violet avait tendance à avoir des teneurs en fibres et en protéines plus faibles que le fourrage seul de *Th. intermedium*, tout en augmentant le rendement fourrager. Ce mélange a triplé la quantité de fourrage disponible à l'automne (Favre et al., 2019).

En tant que culture à double usage, une série d'effets du mélange de légumineuses sur le rendement en grains de *Th. intermedium* a été documentée. Certaines expériences ont montré des rendements en grains plus faibles dans un mélange avec de la luzerne (*Medicago sativa* L.) ou du trèfle violet (*Trifolium pratense* L.) que dans une culture pure de *Th. intermedium* (Favre et al., 2019; Mårtensson et al., 2022; Pinto et al., 2022; Tautges et al., 2018). D'autres expériences ont montré des rendements en grains similaires dans les mélanges avec de la luzerne, du trèfle d'alexandrie (*Trifolium alexandrinum* L.), du trèfle du Caucase (*Trifolium ambiguum* M. Bieb), du mélilot officinal (*Melilotus officinalis* L.), du trèfle blanc (*Trifolium repens* L.) par rapport à la culture pure (Dick et al., 2018; Pinto et al., 2022; Reilly et al., 2022; Tautges et al., 2018). Ainsi, l'interaction entre les espèces au sein du mélange devrait être étudiée pour favoriser les relations de complémentarité et réduire les relations de concurrence. En outre, les légumineuses sont bien connues pour leur fixation biologique de N_2 atmosphérique, ce qui peut réduire les besoins en engrais azotés synthétiques. Dans l'étude de Mårtensson et al. (2022), la culture en mélange de *Th. intermedium* avec de la luzerne a fourni de meilleures conditions de croissance en termes d'azote et d'état hydrique, ce qui pourrait aider à faire face à des périodes de sécheresse plus extrêmes attendues du fait du changement climatique. Tautges et al. (2018) ont également comparé deux systèmes de production de *Th. intermedium*, soit en association avec de la luzerne soit fertilisé chimiquement. Bien que l'absorption en nutriments soit similaire entre les deux systèmes, une baisse moins importante de rendement en grains avec le temps a été identifiée pour la culture en association qui a pu faciliter l'acquisition des nutriments par la culture.

4.6. Méthode de réduction de densité

Comme le soulignent Lanker et al. (2019), il est nécessaire de mener des recherches pour trouver des techniques de gestion qui aident à maintenir le rendement en grains avec le vieillissement du peuplement de *Th. intermedium.* Plusieurs processus ont été supposés pour expliquer cette baisse de rendement, notamment une concurrence intraspécifique accrue pour la lumière ou les nutriments avec une densité de talles plus élevée (Jungers et al., 2017; Tautges et al., 2018). La modification de la qualité de la lumière au niveau du bas des tiges pourrait réduire l’initiation des tiges reproductrices ou déclencher le syndrome d’évitement de la lumière (Jungers et al., 2017).

L’éclaircissement mécanique des peuplements et le brûlage de la paille et des chaumes après la récolte des grains ont été utilisés avec succès par le passé aux États-Unis pour les variétés fourragères de *Th. intermedium.* Le rendement en grains a pu être maintenu sur cinq années dans les cultures de production de semences fourragères (Canode, 1965). Récemment, différentes pratiques de gestion visant à accroître la pénétration de la lumière dans le couvert végétal ont été testées, telles que l’espacement entre les lignes de semis (Hunter et al., 2020a), les méthodes de suppression des plantes entre les lignes de semis (i.e., binage ou application d’herbicides ; Law et al. (2020); Pinto et al. (2021); Bergquist et al. (2022)) ou les méthodes de suppression sur l’ensemble de la parcelle par brûlage et fauche (Bergquist et al., 2022; Hunter et al., 2020a; Pinto et al., 2021). Des résultats peu concluants ont été observés. D’une manière générale, ces pratiques agronomiques auraient le potentiel d’augmenter les rendements, mais pas d’empêcher la baisse des rendements avec l’âge du peuplement. En outre, ces opérations à l’échelle de la parcelle peuvent augmenter la fertilité des talles et des épis, mais elles sont souvent associées à une réduction d’autres composantes du rendement telles que la densité des épis. Cela induit une augmentation effective du rendement au sein de la ligne de semis sans augmenter le rendement au sein de de l’ensemble de la parcelle (Pinto et al., 2021).

3

Cadre conceptuel et objectifs de la thèse

L'objectif général de cette thèse est d'évaluer les performances de *Thinopyrum intermedium* comme culture céréalière pérenne sous différentes conditions environnementales (Figure 3.1). Cet objectif a été divisé selon trois principaux axes de recherche.

Le premier axe était dédié à l'étude de son écologie fonctionnelle. Pour ce faire, diverses gestions agronomiques ont été testées au champ (e.g., date de semis, écartement entre les lignes de semis, fertilisation azotée, association aux légumineuses, régimes de fauche) au sein de la ferme expérimentale de l'ULiège - Gembloux Agro-Bio Tech (i.e., région limoneuse de Hesbaye). Ces essais étaient généralement implantés pour une durée de quatre ans. Le développement de la culture lors des premiers jours après semis (i.e., phase d'établissement) a également été testé en conditions contrôlées et comparé à une céréale annuelle (*Triticum aestivum* L.). Ces différentes conditions environnementales ont contraint le développement de la culture et ont permis l'étude de différents traits fonctionnels.

- Hypothèse 1 (H1) : En tant qu'espèce pérenne, une partie importante des ressources devrait être allouée vers ses structures pérennes (Wagoner, 1990) telles que les racines, les rhizomes ou encore la base des tiges.
- Hypothèse 2 (H2) : L'investissement plus important de l'espèce dans son système racinaire par rapport au blé annuel (Sprunger et al., 2018a) devrait également être observé en phase d'établissement.

Le second axe était dédié à la compréhension des besoins azotés de la culture afin de déterminer la fertilisation optimale à apporter. Différentes doses d'azote et moments d'applications ont été testés au champ pendant quatre années de culture.

- Hypothèse 3 (H3) : En tant qu'élement essentiel à la productivité des plantes cultivées (Gastal et al., 2015), l'apport d'azote devrait améliorer le développement végétatif et le potentiel de rendement en grains et en fourrage de l'espèce.

Finalement, le dernier axe était dédié à l'étude des services de production de l'espèce tant pour l'alimentation animale qu'humaine au sein des différents essais au champ. L'étude de la production céréalière visait à évaluer les différentes composantes du rendement en grains et le rendement en résultant. L'étude de la production fourragère avait pour objectif d'évaluer son potentiel, tant en termes de rendement qu'en termes de valeurs nutritives. Ainsi, les productions de biomasses ont été évaluées sous différentes valorisations (i.e., double usage grain-fourrage ou simple usage fourrager). Tandis que les valeurs nutritives ont été évaluées sur l'ensemble de la saison culturale. Pour ce faire, un travail de calibration et de validation de la méthode d'évaluation des paramètres fourragers par spectrométrie infrarouge a été réalisé.

Performances d'une céréale pérenne, *Th. intermedium*.

- Hypothèse 4 (H4) : L'espèce étant caractérisée par une histoire de sélection récente (DeHaan et al., 2018), ses rendements céréaliers sont supposés être moins élevés mais pourraient partager des mécanismes similaires aux céréales annuelles.
- Hypothèse 5 (H5) : En tant que graminée pérenne, la majorité de sa productivité primaire nette est dirigée vers des structures non reproductives (Culman et al., 2023), ce qui pourrait assurer une production fourragère intéressante.
- Hypothèse 6 (H6) : Par une stabilité au fil des années de la production de biomasse aérienne et de la qualité du fourrage récolté (Culman et al., 2023), une gestion à simple usage fourrager pourrait être envisagée.
- Hypothèse 7 (H7) : En réduisant la surface photosynthétique utile à son développement (Culman et al., 2023), l'exploitation fourragère de la culture pourrait réduire son allocation reproductive.

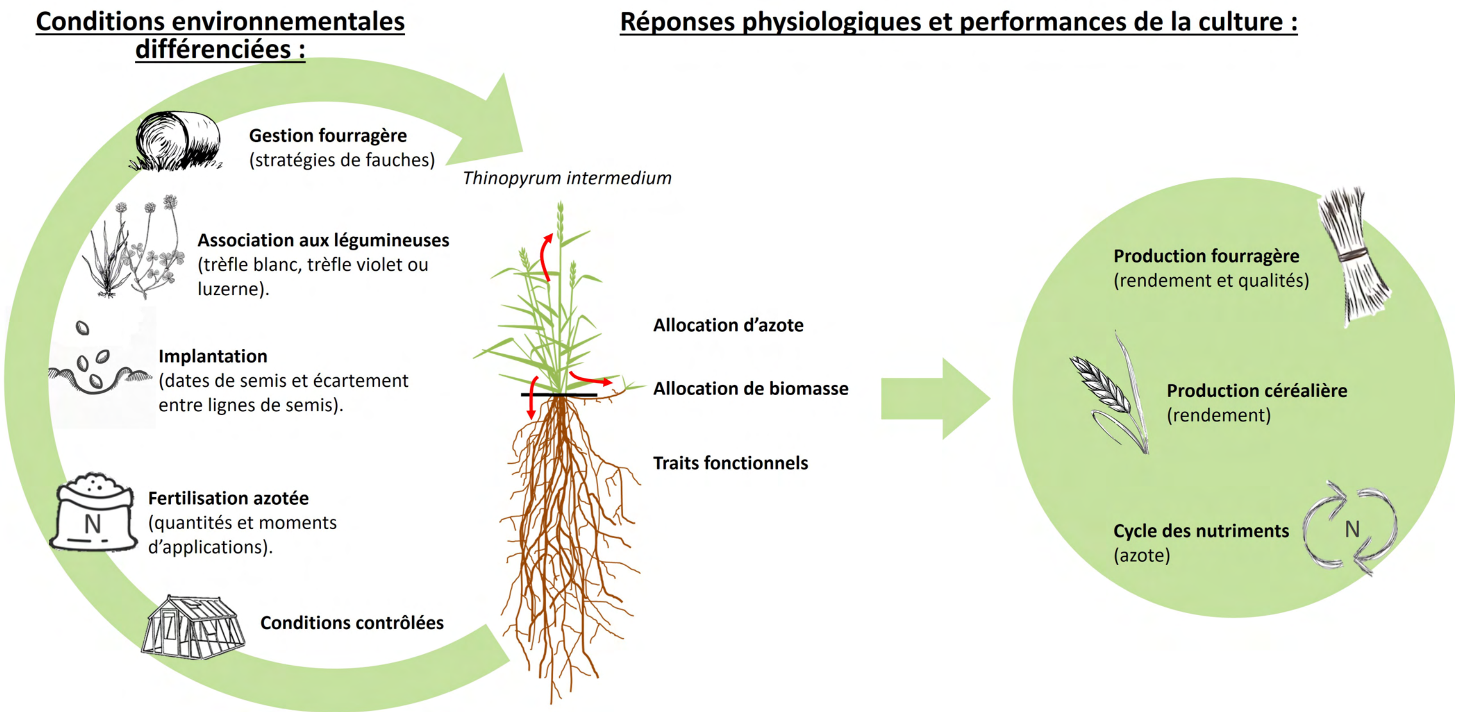

FIGURE 3.1 – Illustration du cadre conceptuel de la thèse.

4

Contribution à l'étude de l'écologie fonctionnelle de *Th. intermedium*

1. Caractérisation du développement en phase d'établissement

Introduction au chapitre

Cette première section de chapitre a été soumise dans le journal 'Agrosystems, Geosciences & Environment' et est en attente d'une réponse de la part des éditeurs.

Celle-ci se concentre sur la caractérisation de différents traits fonctionnels de la culture en phase d'établissement (i.e., quelques jours après semis). Pour ce faire, *Th. intermedium* et *Triticum aestivum* (blé d'hiver) ont été cultivés en rhizobox afin de suivre leurs développements aérien et souterrain. L'étude de ces traits fonctionnels apporte une réelle contribution à la caractérisation de la performance de *Th. intermedium* par rapport au blé d'hiver lors de sa phase d'établissement. Actuellement, très peu d'informations existent sur la caractérisation de l'écologie fonctionelle de l'espèce en cours de domestication.

Thinopyrum intermedium showed a slower establishment phase compared to winter wheat in controlled environment.

Fagnant L.[1*], Delaplace P.[2], Delory B.[3], Dumont B.[1]

[*]Corresponding author : laura.fagnant@uliege.be ; +3281622141

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

[2]Uliege – Gembloux AgroBio-Tech, Plant Sciences Axis, Plant genetics lab., Passages des déportés 2, 5030 Gembloux, Belgium. pierre.delaplace@uliege.be

[3]Environmental sciences group, Copernicus institute of sustainable development, Utrecht University, Heidelberglaan 8, 3584 CS Utrecht, The Netherlands. b.m.m.delory@uu.nl

Credit authorship contribution statement

P.D., B.Du. : supervision, conceptualization, planning of the experiments, formulation of research goals and aims, development and design of methodology.
L.F. : formal analyses (statistical and mathematical), interpretation of result, data presentation and visualization, writing – original draft preparation.
B.De. : formal analyses (statistical and mathematical).
P.D. : help provided for data presentation and visualization.
B.De. : development and design of methodology, contributed to the interpretation of result.
P.D., B.De., B.Du. : critical review, commentary and revision, validation.
Note : the samplings were perfomed by Claude Davaine, Emeline Deguide, Louise Delanglez, Alix Hubaux and Tiphaine Perrot.

Keywords

Perennial grain, Kernza®, Establishment, Root system.

Core ideas

- *Thinopyrum intermedium* had a preferential investment in roots compared to wheat in the establishment phase
- A slow establishment phase was observed for *Th. intermedium*
- *Th. intermedium* exhibited traits associated with a resource conservation

strategy

- The competitive advantage of *Th. intermedium* in harsh field conditions can compromise its field establishment

Abstract

Thinopyrum intermedium is currently proposed as a perennial grain crop benefiting from raising interest through its environmental performances. As a new crop, little attention was paid to the understanding of the eco-physiological regulations of its growth cycle. We conducted an experiment in growth chambers to evaluate the aboveground and belowground development of *Th. intermedium* in comparison to annual wheat during the first days of growth after sowing. The dynamic of growth was faster for wheat, in line with a strategy of rapid acquisition of resources and a quicker colonization of its environment compared to *Th. intermedium*. In contrast, the latter had a preferential investment in roots that could reflect an adaptation from nutrient-poor environments to optimize soil resource acquisition. These insights highlighted a slow establishment phase for this new crop, which may lead to difficulties during the field establishment such as weed competition but could enable it to withstand stressful conditions in terms of soil resources.

1.1. Introduction

Thinopyrum intermedium (Host) Barkworth & D.R. Dewey, also known under the trade name Kernza®, is a wheat relative (Mahelka et al., 2011), which is attracting growing interest because of its multiple roles. It produces grain for human consumption and forage for livestock, while providing various ecosystem services. Indeed, as a perennial grass, the extended belowground activity and the year-round soil cover is beneficial for soil protection, soil fertility or water quality (Culman et al., 2013; Duchene et al., 2020). The root system of *Th. intermedium* can be as deep as three meters (Dehaan and Ismail, 2017). The biomass of its coarse and fine roots was estimated to be three to twelve times greater than that of wheat (Sprunger et al., 2018a). This leads, in field, to 90 % of soil colonized by roots within the first 60 cm of the soil compared to 60 % for annual cereals (Duchene et al., 2020). In the study of Sprunger et al. (2018a), *Th. intermedium* allocated 23 to 50 % of its total biomass to roots compared to 10 % for wheat. Although great progress has been achieved through breeding (Bajgain et al., 2022), the crop is still under domestication. Its grain yield potential remains low compared to annual counterparts, with a maximum of 1 t ha^{-1} (Fagnant et al., 2024) as its forage production is mainly made up of the summer straw (Culman et al., 2023).

As highlighted by Duchene et al. (2021), little attention was paid to the understanding of the eco-physiological regulation of *Th. intermedium*'s growth cycle. Measurement of plants' functional traits can help explain their individual responses to biotic and abiotic factors through a trade-off between growth and survival. This approach is useful to identify synergies and trade-offs among market and non-market services (Duru et al., 2015; Freschet et al., 2021). Deriving from previous theoretical and empirical works (see for example Mooney (1972) or Grime (1974)), variation in plant functioning can be linked to an economics spectrum ranging from a resource 'acquisitive' to a resource 'conservative' strategy. These resource economics traits have consequences for plant performance across the continuum of low to high levels of resources inducing a trade-off between 'slow' vs 'fast' return on investment (Reich, 2014). Plant species on the fast side of the plant economics spectrum are usually characterized by greater photosynthetic activity and respiration rates, higher leaf nutrient concentration (e.g., high N content), and lower leaf mass per unit leaf area (which is the inverse of the specific leaf area). Plant species located on the slow side of the gradient, however, have the opposite strategy and build longer-lived leaves that are better defended with a lower nutrient concentration and greater leaf mass per unit area (Weigelt et al., 2021; Wright et al., 2004). In addition to leaf traits, other traits linked to seed production, fine root construction, and development and maintenance of symbiotic relationships with mycorrhiza are undoubtedly important for plant fitness (Wright et al., 2004). Contrary to leaf traits, traits related to the fine root construction are multidimensional (Kramer-Walter et al., 2016). Using global root trait data, Bergmann et al. (2020) identified two main axes of root trait variation between plant species. The first axis, referred to as the collaboration gradient, represents the trade-off between species with 'do-it-yourself' (DIY) and 'outsourcing' strategies. While DIY species build long but fine roots (low root diameter, high specific root length) to explore the soil and take up resources, outsourcing species outsource resource uptake to AMF by building thicker roots with a larger cortex fraction that are better suited to host AMF. The second axis, referred to as the conservation gradient, represents a trade-off between fast and slow return on investment. While species on the fast side of the root conservation gradient are characterized by a higher than average root N concentration, species on the slow side of the gradient have a higher than average root tissue density (Bergmann et al., 2020; Weigelt et al., 2021). Together, the collaboration and the conservation gradients explain 77 % of interspecific root trait variation and form the so-called root economics space (Bergmann et al., 2020; Weigelt et al., 2021).

As highlighted by Roumet et al. (2006), annuals generally occur in disturbed habitats and have functional traits enabling a rapid exploitation of resources, which is crucial to grow fast and to complete their life cycle in a short period of time. By contrast, perennial species have functional traits reflecting longer-lived tissues associated with persistence and defense probably originating from an adaptation to survive and to monopolize space in environments where the competition is strong (Roumet et al., 2006). Some evidence of resource conservation of *Th. intermedium* was highlighted in different studies. Duchene et al. (2021) suggested that *Th. intermedium* belongs to the slow-growing and more 'conservative' plants under French and Belgium temperate conditions through the higher accumulation of growing degree days until flowering compared to earlier plants (e.g. *Lolium perenne* L. or *Festuca pratense* Huds.). These later flowering species are generally characterized by taller stands, longer leaf lifespan, lower specific leaf area, lower leaf N concentration and relative growth rate. More recently, through the establishment of its N dilution curve, Fagnant et al. (2023) found out that N requirements of the crop were much lower compared to conventional annual crop such as *Triticum aestivum* L. (wheat) or *Zea mays* L. (maize). This trait is consistent with the high N use efficiency reported for this species (Sprunger et al., 2018a). Furthermore, a decrease in the N amount (i.e., mass of N per hectare) of the aboveground biomass during the second phase of growth was also observed and can be related to the long-term survival strategy of the crop, with reduced resource allocation to seeds and substantial investments belowground (Fagnant et al., 2023). In this regard, Duchene et al. (2020) hypothesized that some observed root traits of *Th. intermedium* could also be linked to a resource conservative strategy, namely the higher tissue density.

In this study, our aim was to compare early growth dynamics (i.e., the first days of growth after sowing), biomass allocation, and above- and belowground functional strategies of *Th. intermedium* with a major annual cereal crop, namely *Triticum aestivum* L. Considering the difficulty of measuring root traits in field, this comparison was conducted under controlled environmental conditions.

1.2. Materials and methods

1.2.1. Selection of species and growth conditions

The perennial grass *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey and its annual counterpart *Triticum aestivum* L. (winter wheat) were grown in a growth chamber under controlled conditions, at the faculty of ULiège –

Gembloux Agro-Bio Tech, Belgium. Crops were grown in adequate conditions, i.e. with air temperature ranging from 20.5 to 22.5 °C, a mean relative humidity of 59 %, and a daylength of 14 h with a light irradiance of 360 µmol m^2 s^{-1}.

The seeds of *Th. intermedium* originated from The Land Institute ; 8^{th} cycle of selection (TLI-C8) with an average seed mass of 13 mg. The average seed mass of wheat (Chevignon variety) was 46 mg. The experiment was conducted until seedling establishment, with plants no longer relying on seed reserves (i.e., fifteen days after sowing – BBCH14 for wheat and BBCH12 for *Th. intermedium* (Meier, 2018)). A completely randomized block design with eight replicates per species were used (i.e., four blocks, with two replicates per block). To facilitate root growth analyses, the two species were grown in rhizoboxes (width : 30 cm, height : 60 cm, thickness : 2 cm) filled with a 5 mm-sieved substrate composed of compost (70 %, v/v) and sand (30 %, v/v) (Alonso-Crespo et al., 2023), and characterized by a density of 1.6 g cm^{-3} at the maximal water holding capacity at the beginning of the experiment. One pre-germinated seed was placed against the transparent window of each rhizobox, 2 cm below the soil surface.

Each rhizobox was watered regularly to keep the substrate at water holding capacity throughout the experiment. Boxes were inclined at a 45° angle with the transparent window facing downwards to allow the roots to grow along the transparent front window. To prevent light from reaching the roots, the transparent window was covered by an opaque plate. The experiment was stopped when roots reached the bottom of the rhizoboxes (i.e., 15 days after transplantation).

1.2.2. Data collection

The root system development of each species was followed non-destructively using image analysis as described in Alonso-Crespo et al. (2023). Briefly, images of roots growing along the front window of the rhizoboxes were taken on the 9^{th}, 10^{th}, 11^{th}, 12^{th} and 15^{th} day after seeding. Image acquisition was done with a digital camera (i.e., Nikon D3400 with the AF-P DX NIKKOR 18-55 m lens, image resolution : 453 dpi) as shown in Figure 4.1. The image analysis pipeline consisted of two steps. First, a convolutional neural network was trained with the RootPainter software to detect roots in the images (Smith et al., 2022). Then, the total visible root length was estimated in each image by analyzing segmented images using the open-access software RhizoVision Explorer v2.0.3 (Seethepalli and York, 2020) using algorithms described by Seethepalli et al. (2021). At the time of image acquisition, the maximum rooting depth was manually measured and the phenological stage of each plant was rated using the BBCH scale for the two species (Meier, 2018). The growing degree days (GDD) were summed during the experiment with a mean value of 21.5 °C.

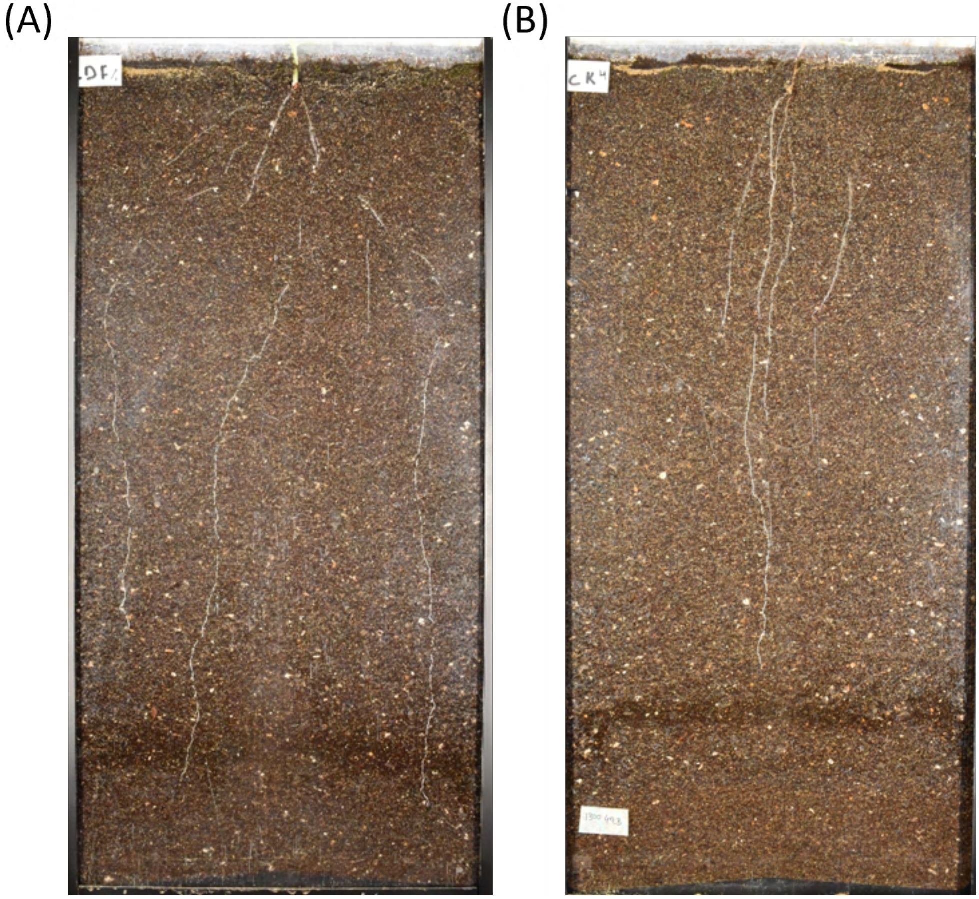

FIGURE 4.1 – Images (scale of 5 :36) of root systems at the end of the experiment (i.e., 15th day after seeding) within rhizobox for (A) wheat and (B) *Th. intermedium*.

At the end of the experiment, rhizoboxes were opened to collect the shoot and root biomass of each plant individual. Shoot and root fresh and dry (dried in an oven at 60 °C until constant weight) weights were measured right after collection. The total leaf area of each plant was obtained by scanning the lamina of fresh leaves on a flatbed scanner. The total leaf area in each image was estimated with ImageJ. The rooting system was separated in three soil layers namely H1 (0-20 cm), H2 (20-40 cm) and H3 (40-60 cm). Roots collected in different soil layers scanned independently on a flatbed scanner (Epson Perfection V800 Photo ; image resolution : 600 dpi) and analyzed using RhizoVision Explorer v2.0.3 (Seethepalli and York, 2020) to determine root length, root diameter and root volume in each image.

Using the data described above, we quantified the following root traits for each species : specific root length (total root length divided by root dry weight), root mass fraction (root dry weight divided by plant dry weight), and root tissue density (ratio of the root system volume obtained and root dry weight).

1.2.3. Statistical analysis

Data analysis was conducted in the R program version 4.1.2 (R Core Team, 2021).

The strength of the linear relationship between the visible root length estimated with image analysis and the root length measured after root system extraction at the end of the experiment was assessed with the *cor_test* function (Pearson's correlation coefficient) from the *rstatix* package. As shown in Figure 4.2, measured and calculated root length were highly positively correlated.

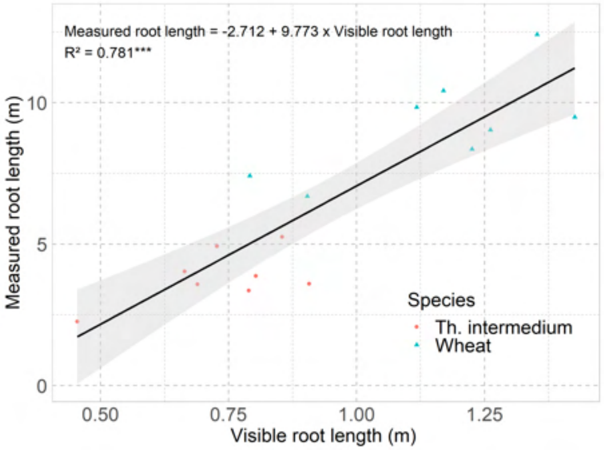

FIGURE 4.2 – Relationship between measured root length computed using root biomass and visible root length computed using image analysis at the end of the experiment. '***' is indicating a statistical significance at p-value<0.001.

The temporal evolution of total root length was modelled using generalized linear mixed-effect model with a gaussian distribution and an inverse-link function from the *glmer* function of the *lme4* package. The maximum rooting depth was modelled using linear mixed-effect model from the *lmer* function of the *lme4* package. The fixed effects were composed of the species treatment and the sum of growing degree days from sowing and their interaction. As these two parameters were measured at multiple time points in each rhizobox during the experiment, a random slope and intercept for each rhizobox was used. Blocks were also considered as a random factor (random intercept). The allometric relationship between the shoot and root biomass at the end of the experiment was tested using a linear mixed-effect model with the *lmer* function from the *lme4* package. The fixed effects were composed of the species treatment and blocks were considered as a random factor.

Leaf area, shoot and root dry weight, root mass fraction, specific root length, total root length, root tissue density, maximum rooting depth and root diameter were analyzed using mixed-effect models, where the species treatment was considered as fixed factor, while blocks were considered as a random factor. Following ANOVA analysis, pairwise comparisons among treatment means were evaluated with the *emmeans* function from the *emmeans* package with a Tukey adjustment for multiple comparisons. Statistical significance was set at 0.05. The *lmer* function from the *lme4* package was used for linear mixed-effect models. They were evaluated to ensure they met model assumptions using the *plotresid* function.

1.3. Results

1.3.1. Ontogenesis

The phenological development of the two species was different, with faster development reported for wheat. The appearance of the second leaf (i.e., BBCH12) was observed at 194 GDD for wheat and at 259 GDD for *Th. intermedium*.

1.3.2. Root system dynamics and development

Dynamic measurements of root front growth (maximum rooting depth) and total root length are presented in Figure 4.3 for the two species. Similar trends were observed for the two variables, with higher values reported for wheat all along the experiment (Figure 4.3A, C). In addition, the coefficients of the temporal evolution of the root front growth were higher, and of the total root length were lower for wheat (Figure 4.3B, D ; Table 4.1). Results translated a faster and denser root development of wheat compared to *Th. intermedium*.

TABLE 4.1 – Intercept and slope coefficients for the temporal evolution of (A) total root length and (C) maximum rooting depth for the two species.

		Intercept		Slope		Equation
		Coefficient *a*	95% credibility interval	Coefficient *b*	95% credibility interval	
(A) Total root length (m)	Wheat	3.97	[3.34 ; 4.60]	-0.01	[-0.01 ; -0.01]	y = 1 / (a + b.x)
	Th. intermedium	8.58	[7.49 ; 9.67]	-0.02	[-0.03 ; -0.02]	
(B) Rooting depth (cm)	Wheat	-2.24	[-5.37 ; 0.89]	0.17	[0.15 ; 0.18]	y = a + b.x
	Th. intermedium	-2.00	[-4.27 ; 0.27]	0.13	[0.12 ; 0.14]	

1.3.3. Biomass production, allocation, and functional traits

The shoot and root parameters measured at the end of the experiment for the two species are presented in Figure 4.4. The dry weight of roots (Figure 4.4A) as well as their length (Figure 4.4B) were higher in wheat compared to *Th. intermedium*. Although we did not find any difference in specific root length between the two species (Figure 4.4C), the root tissue density was higher for *Th. intermedium* and the mean root diameter was higher for wheat (Figure 4.4D, E). Focusing on shoot parameters, *Th. intermedium* had lower shoot dry weight and leaf area than wheat (Figure 4.4F, G). The root mass fraction (i.e., proportion of the biomass of roots to the entire plant biomass) of *Th. intermedium* was higher than the one observed in wheat (Figure 4.4H).

Root proportion (i.e., root biomass within the soil layer divided by the total root biomass) within the different soil layers is presented in Figure 4.5. Through a slower root system establishment (Figure 4.3), the root proportion of *Th. intermedium* is higher (i.e., 77 %) in the first cm of soil compared to wheat (i.e., 67 %) as the opposed situation was observed under deeper soil layers (i.e., 20-40 cm and 40-60cm ; Figure 4.5).

The allometric relationship at the end of the experiment between shoot and root biomass is shown in Figure 4.6 for the two species. Shoot and root biomass were higher for wheat. However, the relationship between shoot and root biomass were not statistically different between species as the slopes have a wide 95 % credibility interval (Figure 4.6).

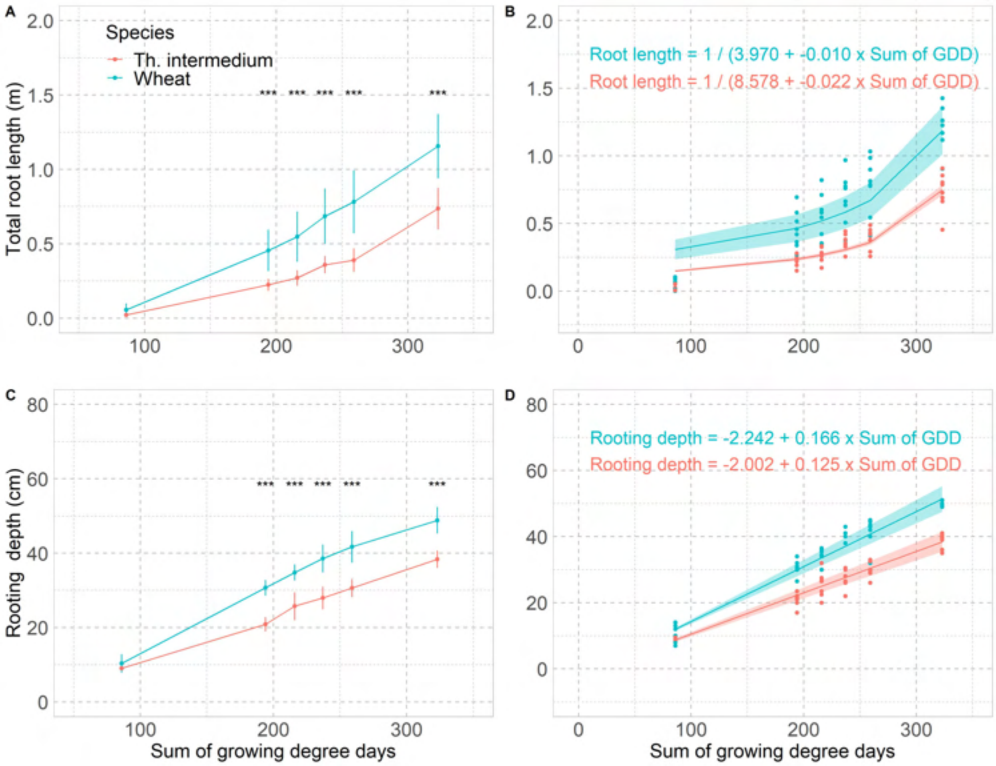

FIGURE 4.3 – Temporal evolution of (A) total root length and (C) maximum rooting depth for the two species. Panels B and D show generalized mixed-effect model fits describing the temporal evolution of (B) total root length and (D) maximum rooting depth. Individual observations are shown as dots. In (A) and (C), statistical differences (post hoc analysis) between species are indicated by “***” (p-value<0.001).

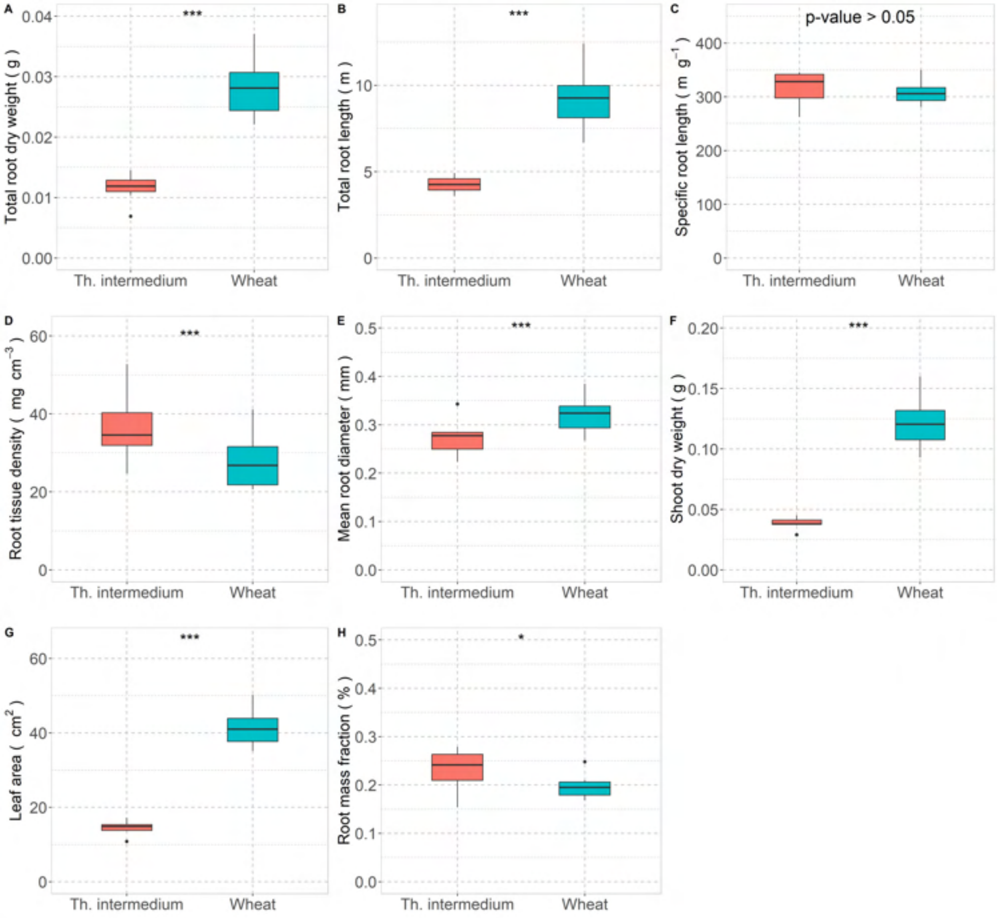

FIGURE 4.4 – Shoot and root parameters measured at the end of the experiment : (A) total root dry weight, (B) total root length, (C) specific root length, (D) root tissue density, (E) mean root diameter, (F) shoot dry weight, (G) leaf area and (H) root mass fraction for the two species. Statistical differences (post hoc analysis) between species are indicated by '*' (p-value<0.05) ; '**' (p-value<0.01) ; '***' (p-value<0.001).

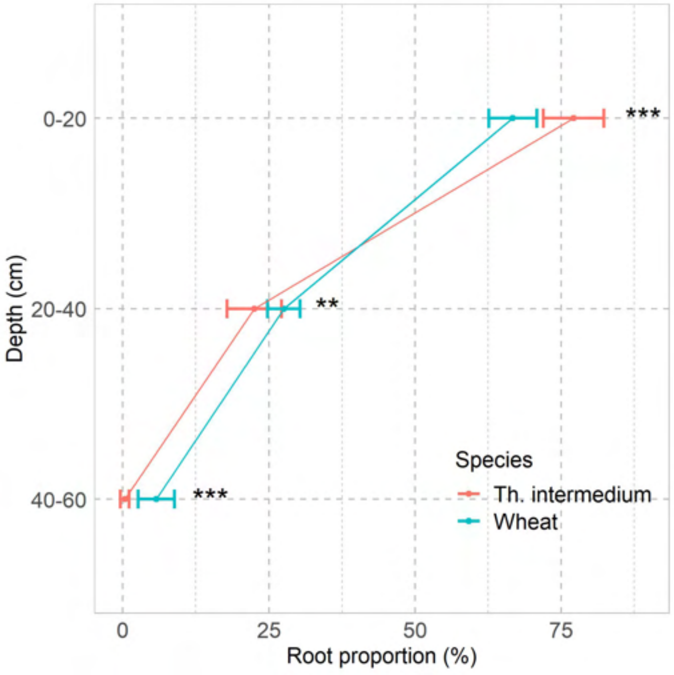

FIGURE 4.5 – Relative proportion of roots in each soil layers for each species. Statistical differences (post hoc analysis) between species are indicated by '**' (p-value<0.01) ; '***' (p-value<0.001).

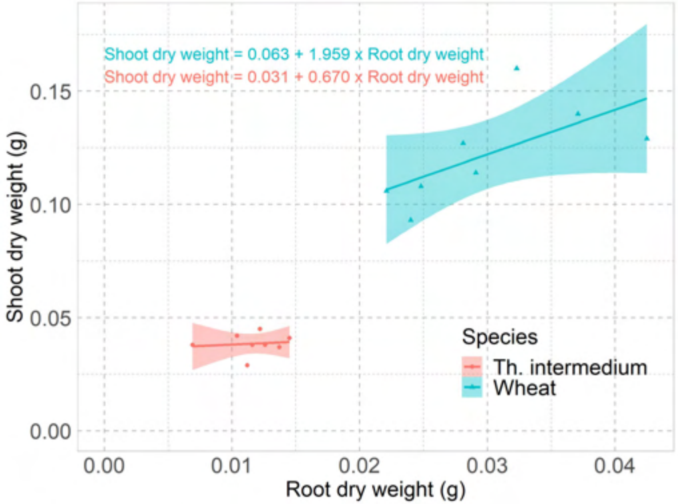

FIGURE 4.6 – Allometric relationship between shoot and root dry weight for the two species at the end of the experiment. The slope 95 % credibility interval was of [0.134 ; 3.807] for wheat and of [-0.866 ; 1.903] for *Th. intermedium*

1.4. Discussion

As highlighted by Freschet et al. (2021), root trait values can strongly differ between plants grown in laboratory and field experiments, as the consequence of different environmental conditions. Under our controlled conditions and without obstacle for root growth, the dynamic of growth was faster for winter wheat compared to *Th. intermedium*. More precisely, the higher relative growth rate of winter wheat led to higher values of total root length, rooting depth, total root dry weight, shoot dry weight and leaf area at the end of our experiment (Figure 4.3 and Figure 4.4). These findings are in line with a strategy of rapid colonization of the environment and acquisition of resources highlighting a 'fast' return on investment into tissue construction (Weigelt et al., 2021; Wright et al., 2004). In contrast, the higher root tissue density of *Th. intermedium* (Figure 4.4D) revealed a 'conservative' strategy, in line with what observed Duchene et al. (2020) in field experiments. This could reflect an adaptation to infertile soils as, in these conditions, slower growth and higher root, stem and leaf tissue densities are associated with greater fitness (Kramer-Walter et al., 2016). The specific root length was similar between species as a higher mean root diameter was observed for wheat (Figure 4.4C, E). This is in contradiction with Duchene et al. (2020) that observed a higher root diameter and a lower specific root length of *Th. intermedium* in comparison with annual wheat or rye in field. They suggested that the less efficient soil exploration of *Th. intermedium* (i.e., lower specific root length and higher root diameter) was compensated by soil resource acquisition through mycorrhizal symbionts as the AMF biomass in the soil increased under *Th. intermedium*. In our study, we didn't differentiate coarse (i.e., transportive roots) and fine roots (i.e., acquisitive roots), leading to difficulties in interpreting these parameters. More dedicated work is therefore needed to conclude on the 'outsourcing' strategy of these two species.

Regarding the relationship between shoot and root biomass at the end of the experiment, dynamics between species are very contrasting. Higher root biomass was translated into a higher shoot biomass for wheat (Figure 4.6). This could be related to the root partitioning within the plant, that was higher for *Th. intermedium* (Figure 4.4H). The greater proportion of biomass into roots could potentially induced a greater proportion of photosynthetically fixed carbon which is respired and partially explain its lower relative growth rate (Poorter et al., 1990). The greater investment in soil exploration of *Th. intermedium* was also highlighted in field experiment where Sprunger et al. (2018a) found out that *Th. intermedium* allocated 23 to 50 % of its total biomass to roots while it was only about 10 % for wheat, leading to a root-shoot ratio two times greater for *Th. intermedium* than for wheat. In their study, Sainju et al. (2017) even reported root-shoot ratio more than eight times greater for *Th. intermedium* compared to spring wheat. The higher proportion of roots most probably reflects an adaptation to nutrient-poor environments, where belowground competition prevails, to acquire more of soil resource (Tilman, 1985). Greater root mass fraction is one of the mechanisms by which plants cope with limitations in soil resources (Poorter, 1989). In contrast, species with a higher amount of reserves in the seed - as it is the case for wheat – can increase the aboveground biomass partitioning of seedlings (Mašková and Herben, 2018). This reflects an adaptation from nutrient-rich environment where fast development of shoots may be highly beneficial to ensure a better light interception that could then represent the limiting resource. However, the poor early vigor of *Th. intermedium*, translated by low shoot biomass, can result in difficulties for its field establishment in comparison to traditional wheat varieties. In practice, this was previously observed in field conditions by Duchene et al. (2023) who reported low *Th. intermedium* biomass during the first establishment year, which resulted in important weed competition.

1.5. Conclusions

The functional traits that were selected to grow in contrasted environments reflected different conceptual strategies set up by crops to ensure their long-term survival. Results gained in this rhizobox experiment conducted during the establishment phase of two crop species globally confirmed that winter wheat is characterized by a strategy of rapid acquisition of resources and a quicker colonization of its environment, compared to *Th. intermedium*. Such a development pattern reflects an adaptation to nutrient-rich environments where an effective strategy would then be to maximize the capture of light through greater leaf development. In contrast, *Th. intermedium* exhibited traits associated with a resource conservation strategy. While its absolute above- and belowground growth rates were lower compared to winter wheat, *Th. intermedium* invest preferentially in its root system. This reflects an adaptation to nutrient-poor environments, through the optimization of soil resource acquisition. While this last trait may provide a competitive advantage in harsh field conditions, it can compromise the field establishment phase of the crop. While some evidence already exists within the literature, the conclusions drawn here were obtained on juvenile plants grown in a controlled conditions and remain to be confirmed within field conditions. Yet, this study contributes to provide new evidence of a resource conversation strategy for *Th. intermedium*, which might be consistent with its well-known perenniality.

2. Caractérisation de l’allocation des ressources au travers de l’âge des peuplements

Introduction au chapitre

Cette deuxième section de chapitre est destinée à être soumise dans un journal international.

Celle-ci se concentre sur la compréhension de l’allocation des ressources, en termes de biomasse et d’azote, au sein des différents organes d’une culture de *Th. intermedium* (i.e., âgée d’un à quatre ans). En effet, aucune étude publiée à ce jour n’a quantifié les allocations, au sein de l’ensemble des organes de l’espèce, de manière dynamique pendant la saison de croissance. Pour ce faire, différents essais expérimentaux au champ ont été suivis au cours de deux années culturales. Cette section apporte de nouveaux éléments concernant l’importance des différents puits végétatifs et reproducteurs au sein de l’espèce.

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

Fagnant L.[1*], Aubry P.[1], Jungers J.[3], Duchene O.[2], Dumont B.[1]

[*]Corresponding author : laura.fagnant@uliege.be ; +3281622141

[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

[3]University of Minnesota, Deptartment of Agronomy and Plant Genetics, St. Paul, MN, United States. junge037@umn.edu

Credit authorship contribution statement
L.F. : conceptualization, planning of the experiments, formulation of research goals and aims, development and design of methodology, carrying out the aboveground samplings, data curation, formal analyses (statistical and mathematical), interpretation of result, data presentation and visualization, writing – original draft preparation.
B.D. : supervision, formulation of research goals and aims.
P.A. : carrying out the belowground samplings, data visualization, writing.
P.A., J.J., O.D., B.D. : critical review, commentary and revision, validation.

2.1. Introduction

Perennial grains are currently being developed to meet some of the sustainability challenges facing agricultural systems. The expected benefits of using perennial grains include reduced soil erosion, nutrient conservation and increased carbon storage (DeHaan et al., 2023). Among perennial grains candidates, the cool-season perennial grass *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey is one of the most worked-on species (Bajgain et al., 2022). Its field cultivation enables the production of both grain for human consumption and forage for livestock. Alongside the production of marketable outputs, the growth of such perennial grain offers ecological benefits through the development of a perennial root system and a continuous soil cover. In mature stands after establishment, the root system can produce up to 7 t of dry matter (DM) ha^{-1} in the upper soil layer (Ogle et al., 2011; Sainju et al., 2017; Duchene et al., 2020). However, the allocation of resources to grains is currently low with a harvest index around 10 %, and a N harvest index ranging from 20 to 50 % (Fagnant et al., 2024; Mårtensson et al., 2022). With low grain yield compared to annual counterparts, effort to improve the grain production have been ongoing since the 2000s (Bajgain et al., 2022; DeHaan et al., 2023). With the increase of seed production through breeding, important questions are raised of whether there is a trade-off between reproductive effort and life span as the life cycle of perennials is based on a balance between reproductive and vegetative growth (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 wild accessions and seed yield improved lineages of *Silphium integrifolium* Michx. and identified changes in N use efficiency. Seed yield improved individuals had greater N allocation to leaves and seeds coupled with a lower storage of N in the stem bases, which could imply higher external N inputs compared to wild accessions. As mentioned by Sakiroglu et al. (2020), understanding above- and belowground biomass production over time, and N allocation and utilization for vegetative or reproductive growth is needed to develop management recommendations for *Th. intermedium* production systems.

In annual grain crops, the pattern of assimilates partitioning changes with the transition from vegetative to reproductive development. During the vegetative development, each leaf begins as a sink and then act as a source of assimilates for the other organs such as new leaves and roots. After the initiation of the reproductive development and up to anthesis, there are three major sinks for assimilates (i.e., initiated leaves, developing stems and sheath tissues) with little assimilates being partitioned to the root system. After anthesis of cereals, the remaining functional leaves and the green spikes act as photosynthetic sources for two competing sinks (i.e., grains and stems), but in the latter stages of grain filling, there is normally an extensive remobilization of carbohydrates 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 resources to belowground and storage structures (e.g., roots, rhizomes, or stem bases) which allows them to efficiently uptake and conserve resources (Roumet et al., 2006; Vico et al., 2016). In grass swards, successful regrowth after defoliation depends upon the mobilization of assimilates stored mainly in the bases of stems to generate the new canopy (Hay and Porter, 2006). Rhizomes are underground plant stems capable of producing the shoot and root systems of a new ramet. As with the roots, these rhizomes are the storage site for carbohydrates and proteins, which can be translocated and used to form new shoots during regrowth after dormancy (Sakiroglu et al., 2020).

Within the question of resource allocation, the issue of N is key, as it is a crucial and limiting element in agricultural production (Gastal et al., 2015). The N requirements of growing tissues of plants 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. However, 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. plants in early spring were met by N remobilization from the old leaves (present on over-wintering tillers) and no N remobilization from the root system was observed. But later in spring, N needs were more fully met by taking N from the soil, even if flower development can result in further internal N translocations, with vegetative tillers providing N to the flowering tillers. 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 of grasses can store carbon (C) and N and use it for leaf development when photosynthetic activity is temporarily reduced by defoliation (grazing or mowing) (Lemaire, 2001). However, this mobilization of reserves for regrowth varies from one species to another (Turner et al., 2007). For *Dactylis glomerata* L., it appears that N reserves play a minor role in the regrowth 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.

For *Th. intermedium*, knowledge of resource allocation between different organs and over time is limited. Previous studies have provided information on the distribution of crop DM including belowground parts, but little is known about the use and mobility of N between the different compartments. 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. Allocation of DM to roots ranged from 23 to 50 % of its total DM, which was higher compared to annual couterparts (Sprunger et al., 2018a; Duchene et al., 2020) with most of the root DM located in the first cm of soil (Sakiroglu et al., 2020). With greater DM of roots but higher 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). Overall, a more pronounced investment in underground structures compared to annual crops represents both a potential for resource storage and remobilization during reproductive growth phases, as well as potentially diverting resources away from seeds depending on resource allocation dynamics (Vico et al., 2016). Sprunger et al. (2018a) observed that the crop 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 amount contained within the aboveground biomass (i.e., above 5 cm of the ground level). This was put in relation to the low seed sink strength of the crop associated with important investment in perennial structures such as roots, rhizomes and stem bases.

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 therefore to quantify the distribution of DM produced by the crop and the distribution of N allocated 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 set up in 2019 and 2021. This characterization of N allocation across plant tissues over the growing season is essential to rationalize and improve crop performance through breeding and field management.

2.2. Materials and methods

2.2.1. 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 4.2. For each experimental site, each plot (i.e., 4 x 8 m) was divided by two to obtain one plot dedicated to grain yield measurement in summer and the other one to destructive sampling during the growing season.

TABLE 4.2 – Detailed information about the experimental sites, their design and management.

		Experimental sites	
Site code :		***BE4***	***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)	Randomized 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 :			
N 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 + autumn mowing	

2.2.2. Aboveground and belowground samplings

Data were 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 BE4 site and the third and fourth cropping years of the BE2 site (Table 4.2). Samples were collected at five different main phenological stages during the growing season ; four reproductive stages rated with the BBCH scale for cereals (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.), as illustrated in Figure 4.7. This BBCH scale was prefered to the system established by Moore et al. (1991) as the standard deviation of the mean stage by count (S_{MSC}) was low. In addition, the phenological development of *Th. intermedium* is closely related to that of annual cereals, with a majority of reproductive tillers.

Aboveground biomass was sampled with 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 weighted to obtain the fresh matter and then oven-dried (72 h at 60 °C) and weighted 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) and the green area index (GAI), without considering spikes, 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 beforehand weighted 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.

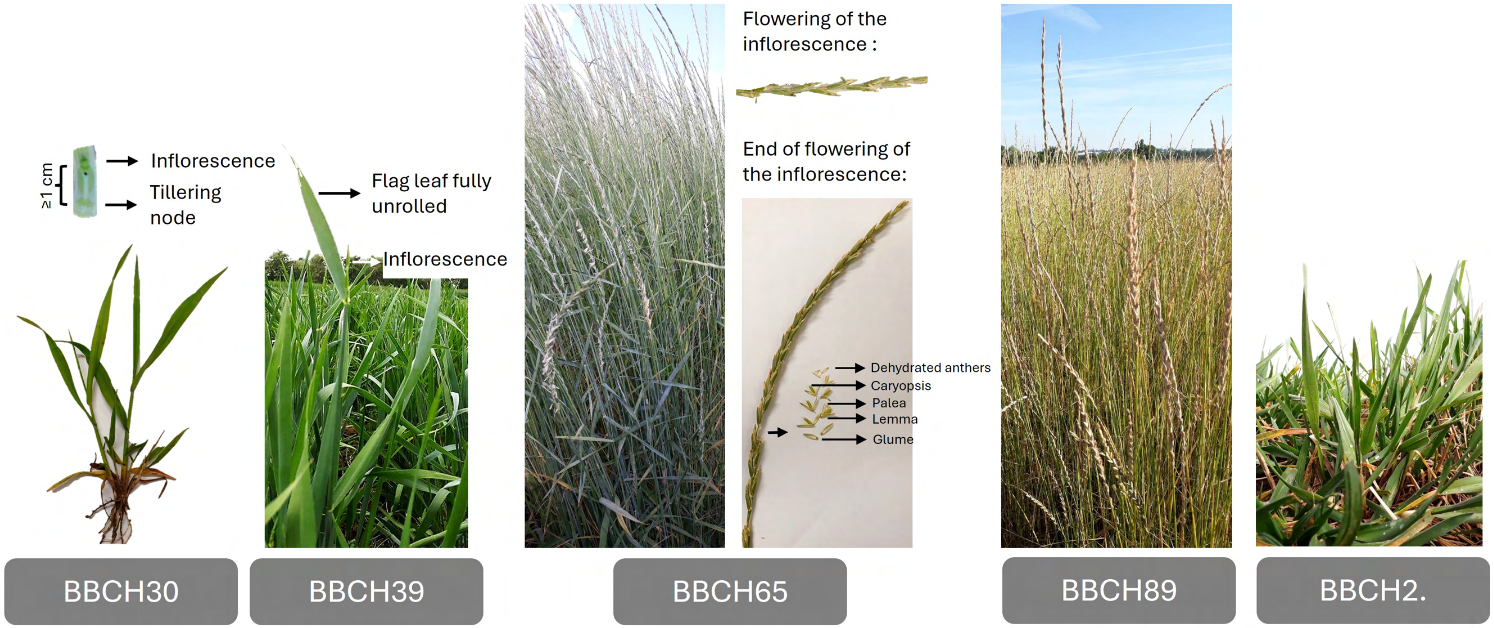

FIGURE 4.7 – Illustration of the different phenological stages (BBCH scale) at which samples are collected.

FIGURE 4.8 – Illustration of a cleaned and fresh sample of belowground and ground-level biomass of *Th. intermedium* from the BE4 experimental site (i.e., stem bases, rhizomes and roots).

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 for each quadrat ; two were collected on the seeding line and two between the seeding lines. Samplings were performed with a metallic auger 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 occurs within the first 20 cm of the soil (Sainju et al., 2017). Indeed, 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 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 4.8. All the samples were then weighted to obtain DM.

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 trial combine harvester to obtain the grain yield on a DM basis and 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.

2.2.3. 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.

2.3. Results

2.3.1. Aboveground and belowground allocation of DM

The production and distribution of crop DM evolves with phenological development (Figure 4.9). 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}. 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 4.9).

During the growing season, the highest DM of leaves was observed at the flag leaf stage (i.e., BBCH39 ; p-value$<$0.001), corresponding to the maximal photosynthetic leaf area (i.e., LAI) (p-value$<$0.05 ; Table 4.3). The highest DM of stems and stem bases was observed at grain harvest, where it represented 82 % of the aboveground DM (p-value$<$0.01 ; Figure 4.9). The GAI (i.e., representing the green area of leaves and stems) was the highest at the flowering stage in 2022 and at the flag leaf stage in 2023 (p-value$<$0.001 ; Table 4.3). Concerning grain yield components, the tiller density at the beginning of the growing season ranged from 349 to 1086 tillers m^{-2}, tiller fertility ranged from 66 to 95 % and the number of spikes at grain maturity ranged from 377 to 603 spikes m^{-2} (Table 4.3). The spikes represented around 10 % of the aboveground DM (comprising stem bases). This resulted in a grain yield ranging from 0.9 to 1.7 t ha^{-1} and a harvest index from 5 to 10 % of the aboveground DM comprising stem bases (Table 4.3). Regarding belowground DM (i.e., roots and rhizomes), it ranged from 2 to 7 t of DM ha^{-1} (Figure 4.9). The effect of phenological development on the root DM was significant only in 2022, with higher DM at the end of the growing season (i.e., BBCH65 or BBCH89 ; p-value$<$0.001 ; Figure 4.9). As observed in Table 4.3, the root mass fraction (i.e., root DM divided by the total plant DM) ranged from 14 to 42 %, with the highest values observed during vegetative stages (i.e., BBCH30 and BBCH2.), and the lowest values during aboveground DM production peaks (i.e., at the end of the growing season or in 2023 ; p-value$<$0.001 ; Table 4.3). The DM of rhizomes represented between 4 to 22 % of the belowground DM and was not influenced by phenological stages except in the first cropping year of the BE4 site. Indeed, the initiation of rhizomes appeared between the flag leaf and the flowering stages that increased their DM during the first growing season (p-value$<$0.001 ; Figure 4.9A).

The experimental site also induced some differences of 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 BE4 site (p-value$<$0.01). At the opposite, the BE4 site had higher rhizome's DM (p-value$<$ 0.001 ; Figure 4.9). An influence of the crop age nested among the two experimental sites was also observed. In the BE4 experimental site comprising the two first years of the crop, the aboveground DM (i.e., leaves, stems and stem bases), as well as the LAI and GAI, increased from the first to the second cropping year (p-value$<$0.05 ; Figure 4.9A ; Table 4.3). In parallel, the tiller density increased until the beginning of the reproductive phase of the second year (p-value$<$0.001), resulting in a higher spike density and grain yield in the second year ($p<0.05$; Table 4.3). In contrast, the allocation of DM to spikes was similar between the two years of the BE4 site (Figure 4.9A). During the first cropping year, we observed an increase of the belowground DM (i.e., roots and rhizomes) inducing higher belowground DM at the beginning of the second cropping year ($p\text{-value}<0.001$; Figure 4.9B). During these two first cropping years (BE4 site), rhizomes represented 20 % of the belowground DM. Focusing on the BE2 experimental site comprising the third and fourth cropping years, rhizomes only represented an average of 8 % of the belowground DM (Figure 4.9B). Once the crop well established, as observed in this site, no significant differences of DM (both above- and belowground) and photosynthetic area were observed between cropping years. However, an increase of stem bases' DM was observed from the third to the fourth cropping year ($p\text{-value}<0.05$; Figure 4.9B). In parallel, a strong decrease of grain yield was observed between the two cropping years ($p\text{-value}<0.001$; Table 4.3), as the harvest index dropped from 10 to 5 % and tiller fertility from 85 to 66 % (Table 4.3).

2.3.2. Aboveground and belowground allocation of N

As with 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 autumn vegetative stage (i.e., BBCH2. ; Figure 4.10). 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 ; $p\text{-value}<0.05$). However, the amount of N in the spikes did not increase after flowering (Figure 4.10). Spikes represented 14 to 35 % of the aboveground N amount (stem bases included). Stem bases represented 25 to 89 % of the aboveground N amount. When an effect of phenological stages was observed (i.e., in 2023 ; $p\text{-value}<0.01$), the N amount of stem bases decreased from the beginning of stem elongation (i.e., BBCH30) to the flag leaf (i.e., BBCH39) stage before increasing at grain maturity (i.e., BBCH89) and being the highest at the autumn vegetative stage (i.e., BBCH2. ; Figure 4.10). 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 4.10). When an effect of the phenological stage was observed, the amount of N in rhizomes was the highest at the autumn vegetative stage (i.e., in 2023 for the BE2 site and 2022 for the BE4 site ; $p\text{-value}<0.05$) and the amount of N in roots was the highest at the end of the growing season (i.e., flowering or grain maturity in the BE2 site ; $p\text{-value}<0.01$; Figure 4.10B). Considering the total amount of N in the crop (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 BE4 in 2023 (i.e., loss of about 30 kg of N ha^{-1}) (Figure 4.10). This was due to the decrease of N in leaves, stems, and roots without any increase in spike N amounts.

The distribution of crop N was influenced by the experimental site. The BE2 site comprising the third and fourth cropping years had higher amount of N in stem bases and roots compared to the BE4 site (comprising the first and second cropping years ; p-value$<$0.01). The amount of N in rhizomes was higher for the BE4 site (p-value$<$0.001). A difference was also observed in 2022, corresponding to the first cropping year of the BE4 site and the third cropping year of the BE2 site, with a higher amount of N in leaves at the beginning of the growing season and in stems for the BE2 site (p-value$<$0.001 ; Figure 4.10). The crop age nested among the two experimental sites also influenced the crop N. Within the BE4 experimental site comprising the two first cropping years, the amount of N in leaves, stem bases, roots and rhizomes increased from the first to the second cropping year (p-value$<$0.05 ; Figure 4.10A). The amount of N within spikes was similar between the first and the second year (Figure 4.10A), although its proportion within the aboveground N amount (comprising stem bases) decreased from 35 to 21 % in the second cropping year. Within the BE2 experimental site, with plants in their third and fourth cropping years, the crop age had no effect on the amount of N in stems, leaves and roots. However, a higher amount of N within the stem bases was observed in the fourth cropping year, coupled with a lower amount of N within the spikes (p-value$<$0.01 ; Figure 4.10B). The amount of N in rhizomes was also higher in the fourth cropping year, but only from the flowering stage (p-value$<$0.05 ; Figure 4.10B).

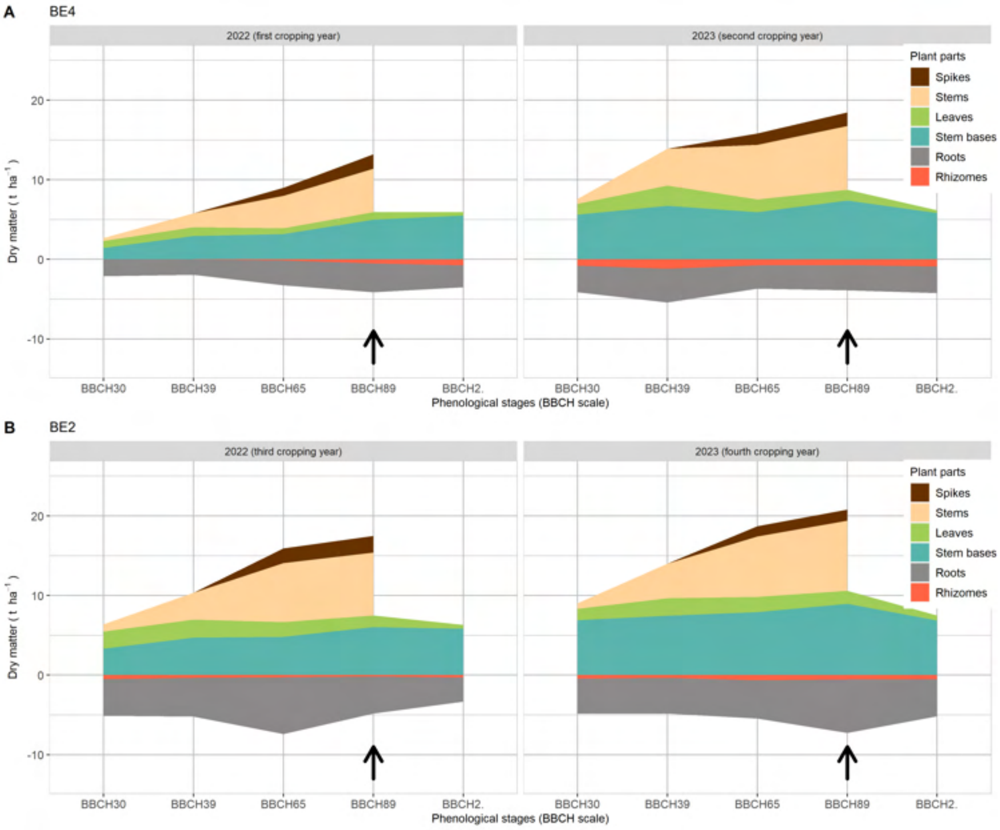

FIGURE 4.9 – DM allocation within *Th. intermedium* plant parts (A) for the BE4 experimental site (i.e., first in 2022 and second cropping years in 2023) and (B) for the BE2 experimental site (i.e., third in 2022 and fourth cropping years in 2023). Arrows represented the grain harvest with an exportation of the aboveground DM (i.e., stems, leaves, and spikes). As belowground plant parts, roots and rhizomes' DM are expressed in a negative value range.

TABLE 4.3 – Grain yield, harvest index, spike and tiller density, LAI, GAI and root mass fraction of *Th. intermedium* for the BE4 and BE2 experimental sites in 2022 and 2023.

		BE4		**BE2**	
		2022 (first cropping year)	**2023** (second cropping year)	**2022** (third cropping year)	2023 (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 (tillers m^{-2})	BBCH30	349±42 A ab	1066±61 B b	1053±111	1086±64 b
	BBCH39	335±42 A ab	826±61 B ab	828±111	689±64 a
	BBCH65	322±42 A a	644±61 B a	960±111	819±64 ab
	BBCH89	511±42 A b	635±61 B a	692±111	820±64 ab
LAI	BBCH30	1.0±0.2 A	2.6±0.3 B b	3.0±0.4	2.9±0.2 b
	BBCH39	1.3±0.2 A	4.3±0.3 B c	2.7±0.4 A	4.5±0.2 B c
	BBCH65	0.9±0.2 B	0.3±0.3 A a	1.5±0.4 B	0.5±0.2 A a
GAI	BBCH30	1.2±0.6 A a	3.2±0.3 B a	3.8±1.0 a	3.7±0.4 c
	BBCH39	2.4±0.6 A ab	8.4±0.3 B b	4.1±1.0 A a	8.1±0.4 B a
	BBCH65	3.9±0.6 A b	6.8±0.3 B b	10.3±1.0 B b	5.5±0.4 A 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 with a letter differ significantly (*p-value*<0.05).Letters in minuscule represent the result of thepost hoc analysis of the effect ofphenological stage and letters in majuscule represent the result of thepost hoc analysis of theeffect of the crop age within each experimental site.

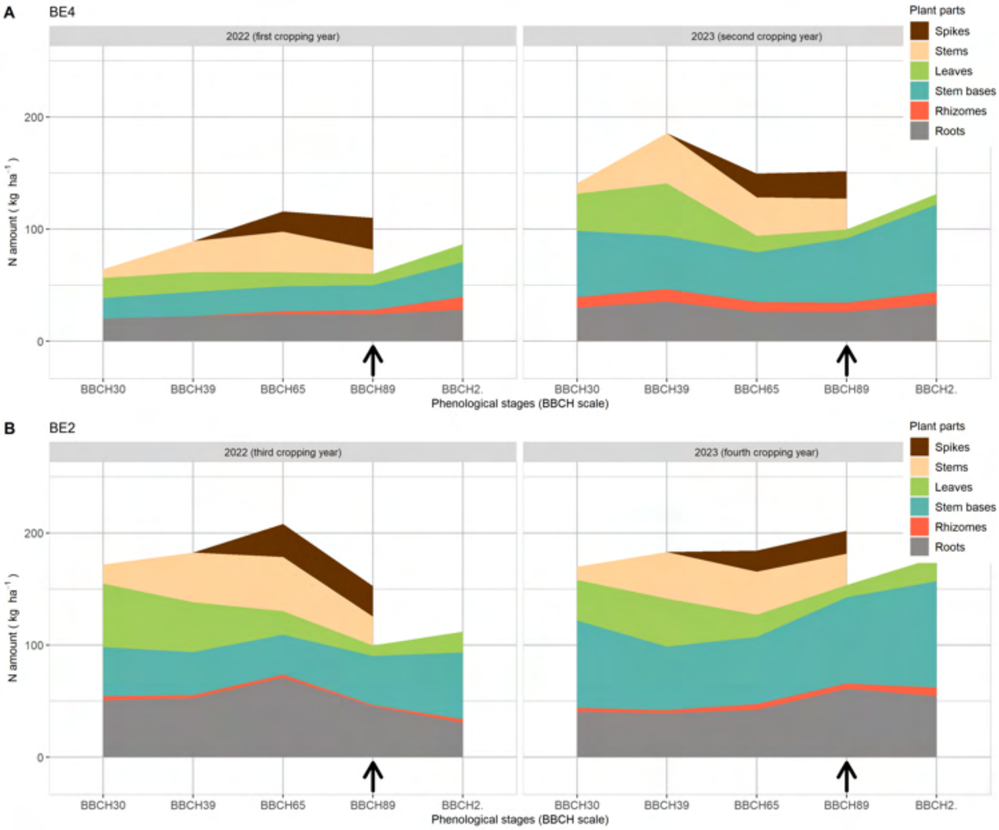

FIGURE 4.10 – N allocation within *Th. intermedium* plant parts (A) for the BE4 experimental site (i.e., first in 2022 and second cropping years in 2023) and (B) for the BE2 experimental site (i.e., third in 2022 and fourth cropping years in 2023). Arrows represented the grain harvest with an exportation of all aboveground biomass except stem bases.

2.4. Discussion

2.4.1. Preserving resources for clonal development

As a cespitose grass with short rhizomes, the stem bases of *Th. intermedium* contain belowground buds for the production of new ramets (i.e., either 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., from 3 to 9 t of DM ha^{-1} and from 36 to 95 kg of N ha^{-1} for regrowing stands excluding the first cropping year) and increased with aging plants (Figures 4.9 and 4.10). Within a given growing season, the DM of stem bases remained relatively constant, although it generally peaked at grain harvest (Figure 4.9). Meanwhile, the increase in the amount of N in stem bases after flowering until the next autumn vegetative stage (Figure 4.10) indicated a translocation of N from leaves, stems or roots, or allocation of new N uptake from the soil pool. Unlike the stem bases, there was no significant increase in the N amount of roots and rhizomes after flowering (Figure 4.10). 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). In our study, the increase of DM in stem bases may also correspond to an increase in water-soluble carbohydrate reserves, as also observed in the basal internodes of *Lolium perenne* (Trethewey and Rolston, 2009) ; this would merit further study.

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 4.9 and 4.10). When the crop was in its two first cropping years (i.e., BE4 site), a higher proportion of rhizomes within the belowground biomass was observed, averaging 20 % compared to only 8 % for older stands (i.e., site BE2). These rhizomes were initiated between the flag leaf and the flowering stage of the first cropping year. Herben et al. (1994) also observed that extravaginal tillers of *Festuca rubra* developed later in the growing season (i.e., in late summer and autumn) compared to intravaginal tillers that develops sylleptically (i.e., at the same time as the growing buds). The intensity of development of extravaginal tillers can be variable and modulated by plant growth conditions. *Th. intermedium* is characterized by a cespitose form which is the result of closely spaced ramet production, allowing a better use of locally abundant resources (Bam et al., 2024). 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. However, some plasticity in growth form under different environmental conditions was observed in other perennial grasses (Bam et al., 2024; Hartnett, 1993). In Bam et al. (2024), *Bromus inermis* Leyss. and *Pascopyrum smithii* (Rydb.) Á.Löve were able to switch to different proportions of extravaginal tillers under different competitive conditions. In our study, the BE4 site was characterized by a low plant and tiller density (i.e., with an average of 73 plants m^{-2} and 380 tillers m^{-2} in 2022 ; Table 4.2, Table 4.3), which may have favored tiller production (i.e., either intra- or extravaginals) thanks to better light penetration within the canopy (Langer, 1963). Also, *Th. intermedium* may have initiated more 'guerilla' tillers (i.e., rhizomes) in this situation, where intraspecific competition was limited compared to more competitive situations with a greater density of plants or tillers (i.e., BE2 site). The ability of *Th. intermedium* to alter its bud placement to favor intra- or extravaginal tiller recruitment in response to competitive evolution should be further studied.

2.4.2. Reproductive allocation

In absolute terms, values of DM and N allocated to spikes remained similar across crop age, with an average of 25 kg of N ha^{-1} for a DM of 1.8 t of ha^{-1} at grain maturity (Figures 4.9 and 4.10). However, the proportion of N allocated to spikes within the plant changed. At grain maturity, it represented 26 % of the total N amount of the plant in the first cropping year (BE4-2022) compared to 10 to 18 % in regrowth years (Figure 4.10). 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 29 to 39 % in regrowth years). Proportionally, the reproductive effort within an establishing crop was higher.

This is emphasized by the values of the harvest index that ranged from 9 to 15 % resulting in a grain yield from 0.9 to 1.7 t ha^{-1} (Table 4.3), 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 4.3), compared to Fagnant et al. (2024) where it ranged from 45 to 84 %. The 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 4.3), reducing competition between tillers within the stand (Fagnant et al., 2024). However, a sharp decline in grain yield was observed in older stands, between the third and the fourth cropping years (i.e., loss of 50 % within the BE2 site ; Table 4.3). This was explained by a decrease in tiller fertility from 85 to 66 % (Table 4.3), a thickening of stem bases, and a lower tiller mortality at the end of the growing season (Table 4.3). A higher proportion of vegetative tillers were maintained, likely diverting resources away from the growing spikes (Yang et al., 2019). 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.

Compared with annual crops, the low grain yields of *Th. intermedium* are also associated with low remobilization of resources to the spikes and grains after flowering. 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). In species such as wheat or rice, 60 to 95 % of grain N at harvest comes from remobilization of N stored in roots and shoots prior to flowering. In contrast, the N storage capacity of shoots (i.e., leaves and stems) of *Th. intermedium* reached a maximum of 92 kg of N ha^{-1} at the flag leaf stage, with a LAI ranging from 3 to 4.5 at the flag leaf stage (Table 4.3). Also, between flowering and grain maturity, the spike DM and N amount didn't statistically increase (Figures 4.9 and 4.10). Resources were shared between vegetative and reproductive organs, and the majority of the allocation of DM and N to spikes were made from the flag leaf to the flowering stages (Figures 4.9 and 4.10). A similar pattern was observed in *Lolium perenne* (Trethewey and Rolston, 2009) where resource allocation to stems (i.e., internodes and leaf sheath) continued after flowering. Trethewey and Rolston (2009) suggested that spikes have sufficient capacity to fill available seeds without contribution from vegetative tissues. In the context of breeding for improved grain yield in *Th. intermedium*, future research will need to identify the ways in which increased resource allocation in the grain is achieved : either by greater remobilization of resources to the spikes at the expense of other vegetative organs, or by mobilizing additional resources.

2.4.3. Root growth and activity drive the N balance of the crop

Roots also accounted for a significant part of plant DM throughout the growing season, ranging from 2 to 7 t of DM ha^{-1} (Figure 4.9), which was also observed in other studies on *Th. intermedium* (Duchene et al., 2020). Roots of *Th. intermedium* within the first 15 cm of the soil contained 20 to 70 kg of N ha^{-1}. As highlighted by Bausenwein et al. (2001), the amount of N in roots is probably more related to the biomass of the rooting system than to the formation of specific storage pools. As root emission is directly linked to the construction of the tiller (Lafarge and Durand, 2011), other studies will need to identify acceptable trade-offs between managing yield over time (associated with tillering activity) and root production and accumulation of a belowground N pool.

The highest amount of N in the whole plant corresponded to the time of the highest observed root DM (Figures 4.9 and 4.10). Conversely, the decrease in root DM at the end of the growing season (Figure 4.10, BE2-2022 and BE4-2023) led to a decrease in the total amount of N in the plant from 34 to 56 kg of N ha^{-1}. However, within this loss of total N in the plant, 22 to 28 kg of N ha^{-1} was identified and was not explained by the reduction in the root system. Since Dobbratz et al. (2023) observed an increase in total soil N from the grain maturity to the autumn regrowth under *Th. intermedium* crop, we suggest with them that there is an influx of organic N into the soil during this period. This influx could be due to root turnover and to the release of N-rich root exudates, or a decreased amount of plant or microbial N immobilization (Dobbratz et al., 2023). 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 cereals, rhizodeposition represents on average 15 % of the total assimilated C, and a median of 14 % of the total assimilated plant N (Wichern et al., 2008). 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 of energy from plant roots, thereby increasing nutrient uptake through enhanced decomposition of soil organic matter.

Finally, we calculated that an average of 60 kg of N ha^{-1} was removed from the field by export of leaves, stems and spikes during summer harvest. This represented 55 % of the total N amount during the first cropping year and 30 to 39 % in regrowth years (Figure 4.10). This 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.).

2.5. Conclusions

Through a relatively low production of rhizomes, *Th. intermedium* is characterized by a conservative phalanx growth strategy, which could 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.

5

Caractérisation des besoin azotés de *Th. intermedium* au travers de sa courbe critique de dilution de l’azote

Introduction au chapitre

Ce chapitre est adapté d'une publication dans le journal 'Field Crop Research' :

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Suite à une meilleure compréhension de l'écologie fonctionelle de la culture, ce chapitre se concentre sur la compréhension des besoins azotés de *Th. intermedium* pour un développement aérien optimal. Pour ce faire, la courbe critique de dilution de l'azote (CNDC) a été établie pour la première fois pour l'espèce grâce à différents essais expérimentaux conduits sous différentes nutritions azotées. Cette courbe représente un outil intéressant pour la détermination du statut azoté de la culture indépendamment des conditions pédoclimatiques tandis que différents éléments, liés à ses stratégies de survie, ont été mis en évidence.

Learning about the growing habits and reproductive strategy of *Thinopyrum intermedium* through the establishment of its critical nitrogen dilution curve.

Fagnant L.[1*], Duchêne O.[2], Celette F.[2], David C.[2], Bindelle J.[3], Dumont B.[1]

*Corresponding author : laura.fagnant@uliege.be ; +3281622141

[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 ; davidc@isara.fr

[3]ULiege - Gembloux AgroBio-Tech, Precision livestock and nutrition laboratory, B- 5030 Gembloux, Belgium Jerome.Bindelle@uliege.be

Credit authorship contribution statement

L.F. : conceptualization, planning of the experiments, formulation of research goals and aims, development and design of methodology, carrying out the samplings, data curation, formal analyses (statistical and mathematical), interpretation of result, data presentation and visualization, writing – original draft preparation.

B.D. : supervision, formulation of research goals and aims, development and design of methodology.

F.C., C.D., O.D., B.D. : help provided for data presentation and visualization, contributed to the interpretation of result.

J.B., F.C., C.D., O.D., B.D. : critical review, commentary and revision, validation.

Keywords

Thinopyrum intermedium, Perennial crop, Intermediate wheatgrass, Nitrogen dilution curve, Nitrogen nutrition index, Nitrogen needs.

Abstract

Context : The perennial grain crop *Thinopyrum intermedium* can provide various ecosystem services and a dual production of grains and forage. Yet, to improve crop management, better knowledges of its physiological behavior and growing habits are required.

Objective : The goal of this study was to characterize *Th. intermedium* N requirements through the evaluation of its response to N fertilization and the subsequent determination of its critical nitrogen dilution curve (CNDC).

Methods : A field experiment was implemented in Belgium during three growing seasons with various N fertilization schemes. Biomass of the different organs and their N contents were measured at specific phenological stages. To estimate the CNDC, a Bayesian hierarchical model was applied on the assembled dataset. The validity of the curve was assessed on an independent dataset including contrasted N situations.

Results : Globally, N fertilization had a positive impact on the DM of leaves, stems and ears ($p\text{-value}<0.05$). The aboveground biomass and N uptake were found maximum with fertilization comprised between 100 and 150 kg N ha^{-1} applied over the entire growing year. At grain harvest, total DM ranged from 7.0 to 16.4 t DM ha^{-1} for a fertilization strategy of 100 kg N ha^{-1}, depending upon the growing season. The N amount of the aboveground biomass was found to decrease during the second phase of the growing cycle. As observed with the proposed CNDC, the aerial N content tended to decrease with the evolution of growing stages and biomass accumulation. Through the low a-coefficient determined for the CNDC, it was confirmed that the crop had reduced need in terms of N nutrition.

Conclusions : The reduced N requirements can be linked to the high nitrogen use efficiency (NUE) and a potential resource-conservative strategy of the crop. This, combined with the observed decrease of the N uptake by the aboveground biomass during the second phase of growth, can be related to the long-term survival strategy of the crop. The latter requires substantial investments in perennial belowground structures coupled with reduced resource allocations to seeds.

Implications : Our study has highlighted that *Th. intermedium* is able to reach a high shoot DM production with low N needs. Our proposed CNDC will be highly helpful to help define N requirements in various pedo-climatic environments and adjust accordingly the soil-crop management, among which the N fertilization. Ultimately, the low N requirements of *Th. intermedium* coupled with a high NUE demonstrated that it could enhance agronomic and environmental benefits.

1. Introduction

The intermediate wheatgrass *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey is developed as a perennial grain crop that can provide ecosystem services including production and preservation services. Previous research has largely focused on its agronomic performances and analyzed both grain and fodder productions (Dick et al., 2018; Jungers et al., 2018; Tautges et al., 2018; Clark et al., 2019; Favre et al., 2019; Barriball, 2020; Hunter et al., 2020a,b). In the meantime, the crop has proven to be valuable in reducing nitrate leaching (Culman et al., 2013; Jungers et al., 2019), or improving soil food webs, carbon pools and sequestration (de Oliveira et al., 2018, 2020; Bergquist, 2019; Sprunger et al., 2019). *Th. intermedium* is characterized by a recent selection history (DeHaan et al., 2018) and its resource allocation to grains is low and variable (Culman et al., 2013; Zhang et al., 2015; Newell and Hayes, 2017). At the same time, its perenniality leads to large resource allocation to the belowground organs composed of short rhizomes and a deep root system to ensure crop continuity (Ogle et al., 2011; Sainju et al., 2017; Sprunger et al., 2018a; Sakiroglu et al., 2020). Consequently, the development of intermediate wheatgrass in cropping system is still impeded by grain yielding capacity and stability, and knowledge gaps about best management practices in fields (Lanker et al., 2019).

To improve yields and crop management, a good description of its physiological behavior and a better understanding of its growing habits are yet required. For instance, recent findings highlighted the ability of using water from deep soil layers and maintaining high water-use efficiency throughout the growing season (de Oliveira et al., 2020; Clément et al., 2022). However, few are known about its N use, whereas crop N management is a key point by being one of the major limiting factors for agricultural productions (Gastal et al., 2015). Some studies have investigated the impact of N fertilization on *Th. intermedium* performances (Jungers et al., 2017; Frahm et al., 2018), without quantifying the soil N supply. Yet, *Th. intermedium* is characterized by a deep and extensive root system, its soil exploration and resource use are better both in space and time through extended growing period (Culman et al., 2013; Jungers et al., 2019; Duchene et al., 2020). This observation may suggest that external sources of N could be minimized without hampering productions of the crop with the benefit of limiting economic and environmental costs of agriculture. Anyhow, there is a need for research devoted to understanding the impacts of N management on crop ecophysiology which will undoubtedly have consequences on agronomical practices and crop N requirements.

To determine the N status of a plant population, the nitrogen nutrition index (NNI) is frequently used. It corresponds to the ratio between the actual N concentration within aerial plant tissues and the critical nitrogen concentration (N_c) required to achieve a non-limiting growth (Lemaire and Gastal, 1997). N_c is derived from the CNDC and represents the minimal N concentration required in shoots to ensure optimal photosynthesis activity and maximize the total aerial dry matter production (W) (Greenwood et al., 1990). The mathematical description of the curve is provided in Equation (5.1) linking N percentage and W using the allometric function proposed by Lemaire and Salette (1984) :

$$\%N = aW^{-b} \tag{5.1}$$

where W is the total shoot biomass expressed in terms of DM (t DM ha^{-1}), %N is the total N content of shoots (% of W), *a* and *b* are coefficients specific to crop parameters. The *a*-coefficient represents the N concentration in the total aboveground biomass at 1 t DM ha^{-1} of W, while the *b*-coefficient influences the shape of the curve (Greenwood et al., 1990; Lemaire and Gastal, 1997; Gastal and Lemaire, 2002; Ziadi et al., 2010; Santana et al., 2020). The CNDC relies on the principle that under non-limiting soil N availability, the N concentration in the aboveground biomass is highly related to the crop growth rate and the DM accumulation. The CNDC has been determined for many cultivated crops including perennial crops (Table 5.1) and has been further used as a reference to discriminate N situations that are over (above the curve, NNI>1, i.e. luxury N consumption) or under (below the curve, NNI<1, i.e. N deficiency) the critical curve, as shown in Figure 5.1, thus driving fertilization rate and timing on crop.

The conventional approach to set-up the CNDC consists firstly in identifying the N_c points and then fit the negative exponential curve to these points Equation (5.1). Different statistical approaches may be used to identify N_c points : (i) analysis of variance and multiple comparisons (Greenwood et al., 1990), (ii) fitting a linear-plateau curve (Justes et al., 1994), or (iii) hierarchical Bayesian modelling (Makowski et al., 2020). Many studies determined N_c points using the simplified statistical method derived from the study of Greenwood et al. (1990). In this approach, ANOVA is first used to identify where variations in W are statistically different under varying N treatments, within each date of sampling. A multiple comparisons analysis is then used to identify the maximal aerial dry matter production (W_{max}), the N content recorded under W_{max} is the critical N_c point. In the event where statistically equivalent W_{max}

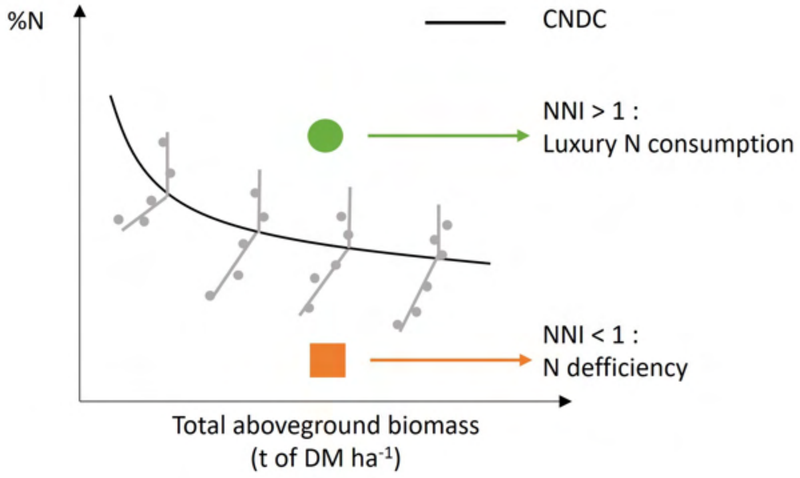

FIGURE 5.1 – Definition and establishement of the CNDC by identifying critical points with the linear-plateau method (Justes et al., 1994).

are reported under two or more N treatments, the lowest N rate is selected as the N_c. However, N_c points selected using this simplified approach might be biased due to potential deficiencies within the experimental dataset such as the N rates might not be sufficient to reach W_{max} (Fernandez et al., 2022). The second method usually requires dataset sufficiently large enough so that a linear-plateau curve can be identified for each observation set, as shown in Figure 5.1. However, this approach remains difficult to implement as the experimental dataset must meet specific statistical criteria, as described in Justes et al. (1994). Finally, more recently, an alternative statistical method based on a hierarchical Bayesian modelling has been proposed by Makowski et al. (2020) to relate the N percentage to the W and analyze concomitantly the uncertainty in the fitted CNDC. The hierarchical Bayesian model simultaneously identifies critical points using the linear-plateau method (Justes et al., 1994) while fitting the negative exponential curve which defines N_c. In principle, this model can estimate CNDC from the direct W - %N pair of observations without classifying limiting and non-limiting N data and without assuming that W_{max} has been reached in all sampling dates (Fernandez et al., 2022). This method has already been successfully used in different study for maize, wheat or tall fescue (Ciampitti et al., 2021b,a; Fernández et al., 2021; Yao et al., 2021). However, the Bayesian hierarchical method might remain subjected to potential inferential bias due to limitations within experimental datasets in terms of quantity and/or quality of the data (Fernández et al., 2021; Fernandez et al., 2022).

The CNDC is a reliable tool to establish diagnoses of the N status of various crop species growing within different climatic and agronomic conditions and further inform on the crop growing habits (Table 5.1). Among else, it has allowed differentiating functionally different plants, such as C3 and C4 plants in the study of Greenwood et al. (1990). The establishment of the CNDC may also contribute to improve the management practices, such as N fertilization. Therefore, to understand growing habits and N requirements of the newly developed perennial grain crop *Th. intermedium*, our objective was to determine the CNDC associated to its growth.

2. Materials and methods

2.1. Experimental sites

To determine the response to N of *Th. intermedium*, a field experiment (BE1) was conducted on the experimental farm of ULiège – Gembloux Agro-Bio Tech, Belgium, using a complete randomized split-plot design (2*8m micro-plots) with four replicates. The first level of randomization is used to assign experimental units to a mowing factor comparing two treatments (not presented in this study). Within these experimental units, different N fertilization treatments (ammonium nitrate granular) were applied on subplots. These treatments differed according to total amount (0, 50, 100 or 150 kg N ha^{-1}) and timing of application (early-spring (BBCH29), mid-spring (BBCH39), and autumn vegetative stage (BBCH2.)) (Table 5.2). Fertilization levels were chosen according to previous studies on N application (Jungers et al., 2017).

Five French additional field experiments (V1-5) were used to provide validation data and assess the reliability of the CNDC established from the main Belgian experimental site (BE1). All detailed information about crop management and experimental designs of the sites used is summarized in Table 5.3.

2.2. Data collection

The data from the analytical site (BE1) used in this study were collected from the second to the fourth growing season after crop implantation. Concerning the validation sites (V1-5), data were collected during the first, the second or both growing season, depending on sites and data availability (Table 5.3). Aboveground biomasses were sampled through a 50x50cm quadrat, cut at 5 cm above the soil surface as frequently performed for forage grasses such as Lemaire and Salette (1984) or Agnusdei et al. (2010). Biomasses were then oven-dried (72h at 60°C) and weighted to obtain DM.

TABLE 5.1 – Coefficients of the CNDC (described in Equation (5.1)) of different cultivated species.

Plant species	***a*-coefficient**	***b*-coefficient**	**Statistical method reference**	**Reference**
C3 crops	5.70	-0.50		(Greenwood et al., 1990)
C4 crops	4.09	-0.50		(Greenwood et al., 1990)
Lolium perenne L. (Perennial ryegrass)	6.36	-0.71	(Justes et al., 1994)	(Gislum and Boelt, 2009)
Solanum tuberosum L. (Potato)	5.37	-0.45	(Greenwood et al., 1990)	(Abdallah et al., 2016)
Triticum aestivum L. (Wheat)	3.90 [2.08 ; 5.47]*	-0.41 [0.20 ; 0.52]*	(Makowski et al., 2020)	(Yao et al., 2021)
Beta vulgaris subsp. *vulgaris* var. alba L. (Fodder beet)	4.9	-0.52	(Greenwood et al., 1990)	(Chakwizira et al., 2016)
Festuca arundinacea Schreb. (Tall fescue)	3.93 [3.59 ; 4.32]*	-0.42 [-0.35 ; -0.49]*	(Makowski et al., 2020)	(Fernández et al., 2021)
Linum usitatissimum L. (Linseed)	4.69	-0.53	(Justes et al., 1994)	(Flénet et al., 2006)
Medicago sativa L. (Alfafa)	[4.6 ; 5.5]	[-0.36 ; -0.29]	(Lemaire and Salette, 1984)	(Lemaire et al., 1985)
Zea mays L. (Maize)	3.49 [3.25 ; 3.78]*	-0.38 [-0.33 ; -0.43]*	(Makowski et al., 2020)	(Ciampitti et al., 2021a)
Miscantus giganteus & *Miscanthus sinensis*	2.70	-0.48	(Greenwood et al., 1990)	(Zapater et al., 2017)
Vitis vinifera L. (Grapevine)	[2.38 ; -3.20]	[-0.17 ; -0.44]	(Lemaire and Salette, 1984)	(Celette and Gary, 2013)

TABLE 5.2 – Timings and amounts (kg N ha^{-1}) of N fertilization treatments of the Belgian experimental BE1 site from 2019 to 2021.

			Splitting (kg N ha^{-1}))				
			2019		**2020 and 2021**		
Code	**Treat-ment**	**Total N dose (kg N ha $^{-1}$))**	**April BBCH29**	**Septembre BBCH2.**	**April BBCH29**	**May BBCH39**	**Septembre BBCH2.**
0+0+0N	1	0	0	0	0	0	0
50+0+0N	2	50	50	0	50	0	0
50+0+50N	3	100	50	50	50	0	50
100+0+0N	4	100	100	0	100	0	0
100+0+50N	5	150	100	50	Not applied		
100+50+0N	6	150	Not applied		100	50	0
0+100+0N	7	100	Not applied		0	100	0
50+50+50N	8	150	Not applied		50	50	50

TABLE 5.3 – Detailed information about the experimental sites, their design and their management.

		Experimental sites					
Site code		**BE1**	**V1**	**V2**	**V3**	**V4**	**V5**
Location							
	Country	Belgium	France				
	GPS Long. (DD)	4.7063	5.1251	5.0920	5.143	5.0419	3.5130
	GPS Lat. (DD)	50.5664	45.4250	45.2746	45.3323	45.4350	45.4638
Soil type		Clay-loam	Loam	Sandy-loam (stony)	Sandy-loam	Sandy-clay-loam (stony)	Clay-loam
Climate							
	Average annual rainfall (mm)	852	881	984	983	927	628
	Average annual min temperature (°c)	7	7.8	6.3	6.3	7.8	6.5
	Average annual max temperature (°c)	14.2	16.5	16.1	16.1	16.5	16.7
Type of experiment		Microplots - randomized split-plot design (4 replicates)	On-farm experiment Strips design (3 replicates)			Microplots - randomized split-block design (3 or 4 replicates)	
Implementation							
	Sowing date (DD/MM/YY)	22-09-17	20-09-17	15-09-18	05-09-17	18-09-18	19-10-17
	Seed population	TLI-C5 : 5th cycle of selection of The Land Institute, Kansas, USA					
	Seeding rate (kg ha^{-1})	20	18			25	18
	Interrow spacing (cm)	25	25	12	20	15	
Field management							
N fertilization (kg ha $^{-1}$)	BBCH30	see (Table 5.2)	50			80	80
	BBCH39		0			40	0
	Weeding	Chemical + mechanical	/			Chemical + hand	
	Crop protection	/	/			/	/
	Post-harvest residue management	Chipping or mowing at 5cm from the ground					
Growing season for data collection		2019, 2020, 2021	2018	2019	2018	2018	2018, 2019

Samples were collected at four different main phenological stages, rated with the BBCH scale (Meier, 2018), namely the stem elongation (BBCH30), the flag leaf (BBCH39), the flowering (BBCH65) and the grain maturity (BBCH89) stage, as illustrated in Figure 4.7. For site BE1 only, ears were always separated from straw biomass. Additionally, leaves were separated from stems in 2020 and 2021. During these two years, the LAI was also measured at three phenological stages (BBCH30, BBCH39 and BBCH65) by collecting leaves on 50 cm of a row in one replicate of each N treatments. They were then laminated with transparent adhesive cover on paper sheets and scanned. These leaves were beforehand weighted to estimate the specific leaf area (i.e., ratio of leaf area to leaf dry mass) to estimate LAI over the three other replicates. Finally, the leaf area ratio (LAR) was calculated by dividing the LAI by the total aboveground biomass.

For all sites, N concentrations (including nitrate) of samples were measured through the Dumas method (Dumas, 1831) ; N contents were quantified individually for each replicate (across all sites, cropping seasons and phenological stages). An exception must be notified for the cropping season 2019, where the sole average samples over the four replicates were available to determine N content at the grain maturity stage for BE1 site.

As a general indication, the apparent N recovery (ANR) efficiency was calculated for each N fertilization treatment (except treatment 1) and each growing season as :

$$ANR = \frac{N_{uptake.treatment_x} - N_{uptake.treatment_1}}{TotalNdose} \tag{5.2}$$

As performed by Sprunger et al. (2018a), the aboveground NUE was also calculated as :

$$NUE = \frac{N_{uptake.treatment_x}}{TotalNdose} \tag{5.3}$$

where the N_{uptake} was the total aboveground N uptake (in kg N ha^{-1}) of the N fertilization treatment at grain harvest and Total N dose (in kg N ha^{-1}) was the total N fertilization applied.

When needed, the four phenological stages were translated into development units (UPVT) (i.e., 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). The corresponding sum of UPVT ($\sum$ UPVT) is of 191 at BBCH30, 413 at BBCH39, 878 at BBCH65 and 1622 at BBCH89, respectively.

2.3. Analysis of the aboveground biomass, N content and N uptake of Th. intermedium (BE1 site)

Analyses of variances (ANOVA) were conducted with the R studio software (R Core Team, 2021). A three-way ANOVA was used, where factors were constituted of i) the growing seasons (year), ii) the N fertilization treatments common to each growing season and iii) the four – or three – phenological stages of the crop at which samples were collected. The total aboveground DM, N uptake, N content, leaf/stem ratio as well as LAR were the analyzed variables. Two-way ANOVA’s were also performed, within each year and for each plant organ, where factors were constituted of i) the N fertilization treatments and ii) the four phenological stages at which samples were collected. The DM, N content and N uptake within plant tissues were the analyzed variables.

Within the different analyses conducted, mixed models were used. The N fertilization, phenological stage and growing season were considered as fixed effect, while replicates as a random effect. Regarding N fertilization effect, N treatments were considered globally, without dissociating timing or amount effect.

When interactions were observed between the fixed effect (fertilization, phenological stage or year), data were separated by the treatments of one factor to analyze the effects of the other factors. Bartlett’s test was used to confirm the homogeneity of variance and Shapiro-Wilk’s test was used to confirm that residuals were normally distributed. Following ANOVA analysis, the post-hoc Student–Newman–Keuls test (SNK test) was used to compare treatment means with a significance level set at 0.05.

2.4. CNDC establishment and validation

2.4.1. The Bayesian hierarchical model to estimate CNDC

To estimate the CNDC, a Bayesian hierarchical model (Makowski et al., 2020) was applied on our consolidated BE1 dataset. In this model the response of W to N content is considered to follow a linear-plus-plateau function. The variability of this function’s parameters across sampling dates is described by a posteriori probability distribution function, estimated using Bayesian method, from which the most probable parameter values of CNDC and their credibility intervals are derived (Makowski et al., 2020), as illustrated in Figure 5.2 and Figure 5.3. The statistical model was assessed using a Markov chain Monte

Carlo algorithm (MCMC) implemented using R (R Core Team, 2021) and its *brms* package (Bürkner, 2017, 2018). As proposed in the study of Bohman et al. (2021), the following non-linear brms model formula was applied :

$$W = min(W_{Max,i} + S_i(\%N_{Plant} - (aW_{Max,i}^{-b})), W_{Max,i}) \tag{5.4}$$

where S_i and $W_{Max,i}$ are respectively the slope of the linear plateau curve and the maximum value of biomass (i.e., plateau) for a given date [i]. min represents the minima function (i.e., the plateau component) and *a*- and *b*-coefficient have the same meaning as previously defined in Equation (5.1). The parameters S and $W_{Max,i}$ included group-level (i.e., random) effects to fit a linear-plateau curve to each sampling date :

$$W_{Max} + S \sim 1 + (1|index) \tag{5.5}$$

where *index* represents the unique level of each experimental sampling date [i].

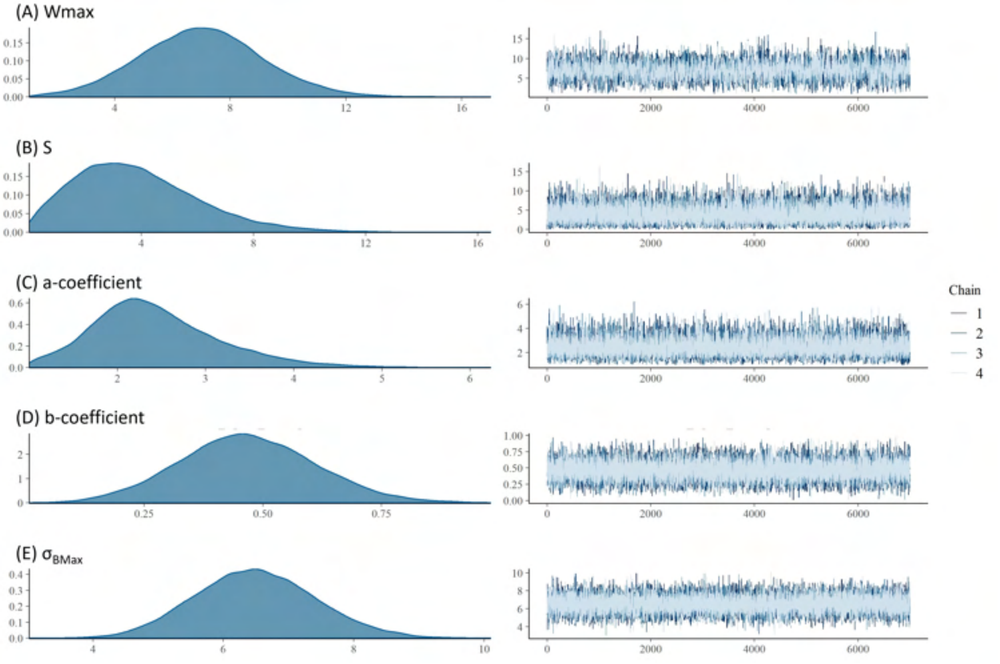

FIGURE 5.2 – Posterior distribution and the post-warmup samples from all 4 Markov chains of 10 000 iterations of (A) W_{max}, (B) S, (C) *a*-coefficient, (D) *b*-coefficient and (E) σ_{BMax},obtained with the Bayesian approach.

2.4.2. Practical considerations and priors setting

Only data from stem elongation (BBCH30) to flowering stage (BBCH65) and with W above 1 t DM ha^{-1} were used. Indeed, as explained in the study

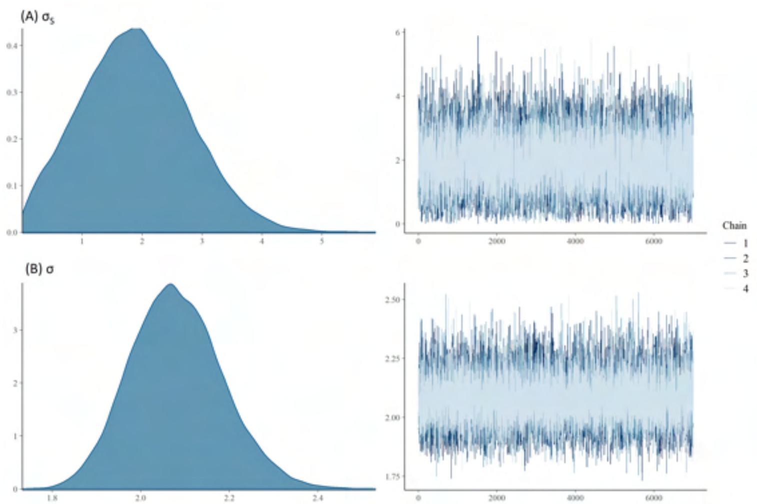

FIGURE 5.3 – Posterior distribution and the post-warmup samples from all 4 Markov chains of 10 000 iterations of (A) σ_S and (B) σ obtained with the Bayesian approach.

of Justes et al. (1994), N dilution would not be significant for low biomass values (less than 1 t DM ha^{-1}) as plant canopy is not closed yet. In addition, the theory explaining decline in N percentage with increasing biomass is mostly restricted to the vegetative period, excluding samplings after the flowering stage (BBCH65) (Greenwood et al., 1990; Justes et al., 1994).

Priors were chosen based on expertise and empirical observations (e.g., summary values from our data set, previously reported values for other species) combined with prior distribution boundaries (e.g., if the range of a prior led to biologically or physically impossible predictions, it was narrowed). Values of priors are reported in Table 5.4.

The MCMC algorithm was run with 4 chains of 10 000 iterations each. A warmup period of 3000 runs was used.

2.4.3. Evaluating uncertainty on parameters and critical N concentration

The *a*- and *b*-coefficients of the CNDC curves were derived from their respective a posteriori distribution. The most probable parameter value was estimated through the median value (centile 0.5) and the 0.025 and 0.975 quantiles were used to determine the 95 % credibility interval (CI).

The uncertainty around the CNDC curve was estimated using the following

TABLE 5.4 – Priors used to fit the CNDC with the hierarchical Bayesian model.

Parameter of the CNDC	**Distribution**	**Boundaries**	
		Lower	**Upper**
a	Normal (3 ; 1)	1	7
b	Normal (0.5 ; 0.15)	0	1
W_{max}	Normal (10 ; 10)	1	30
S	Normal (4 ; 3)	0	infini
σ_{Rmax}	Normal (7 ; 1)	'$-\infty$'	'∞'
σ_s	Normal (2 ; 1)	'$-\infty$'	'∞'
σ	Student's t (3 ; 1 ; 0.1)	'$-\infty$'	'∞'

procedure. The a- and b-coefficients of the 1000 final runs of each of the 4 chains were used to generated CNDC curves. Curves were calculated for a set of discrete values of W ranging from 1 t DM ha^{-1} to the maximum observed value in the experimental data set. From the population of CNDC curves, quantiles 0.025, 0.25, 0.75 and 0.975 were calculated to determine the 50 % CI and 95 % CI. As the estimation of a- and b-coefficients is performed concomitantly by the Bayesian model, this approach allows to account for their correlation and its impact on the generated CNDC curves (Dumont et al., 2014).

2.4.4. Validation of the CNDC (V1-5 sites)

The dataset from validation sites (V1-5) was used to assess the validity of the curve and confirm that it allows to properly distinguishing 'limiting' and 'non-limiting' N situations according to their biomass and N content. To discriminate situations within the validation sites, the following procedure was applied.

At each phenological stage, a one-way ANOVA was performed to determine if statistical differences existed in W and N percentage between sites. When statistical differences were reported, a post-hoc test was performed to group results. The least significant difference (LSD) at the 0.05-significance level (Chakwizira et al., 2016) was calculated to compare and rank means of W and N percentage samples.

Discrimination of the datasets into two groups was made as follows. Samples that were not significantly different from the lowest biomass, were classified as 'limiting' N situations, while samples that did not significantly differ from the highest biomass sample, were classified as 'non-limiting' N situations. As many points were not categorized, additional information provided by field experts was required for the validation sites : sites with high N fertilization (80 and 120 kg ha^{-1} in the spring) and known has being non-water limited were considered as 'non-limiting' N situations (V4, V5) ; sites with relatively low N fertilization (50 kg ha^{-1} in the spring), with shallow and stony soils or with a high weed competition were considered as 'limiting' N situations (V1, V2 at BBCH30 and V3 at BBCH39).

3. Results

3.1. Impact of N fertilization on crop growth and nutrient uptake (BE1 site)

3.1.1. Evolution and partitioning of DM in the aboveground biomass

Significant interactions were found between the fixed factors, namely the growing season (year), the fertilization treatment, and the phenological stage (Table 5.5). Therefore Table 5.8 presents detailed results of aboveground biomass within each year and each plant organ (when available). Leaf/stem ratio and LAR are presented in Table 5.6 and Table 5.7).

As expected, the aboveground DM production generally increased along the crop cycle. A sharp increase is observed between the stem elongation (BBCH30) and the flag leave stage (BBCH39), followed by a lower increase of the total aboveground DM until the grain maturity stage (BBCH89). The cumulated aboveground DM of the different plant organs in relation with the development stages is illustrated in Figure 5.4 for the 50+0+50N fertilization level (treatment 3), which was found to best match the plant N requirements (see section 3.2 and Figure 5.8). The total aboveground biomass was found to be highly variable between growing seasons. It reached, at BBCH89 ($\sum$UPVT of 1622), 16.4 t DM ha^{-1} in 2019, only 7.0 t DM ha^{-1} in 2020 and 10.3 t DM ha^{-1} in 2021 (Figure 5.4), indicating the highest final production level in 2019 and the lowest in 2020 (p-value$<$0.001).

Focusing on biomass production of the aboveground organs, the analysis indicated a significant effect of phenological stage. The weight of leaves is generally the highest at BBCH39, before gradually decreasing until BBCH89. Concerning stems, the biomass peak is observed at the flowering stage (BBCH - 65), except in 2021 where the increase was reported until BBCH89 for some N treatments (Table 5.8). The biomass of ears was systematically found to statistically increase between BBCH65 and BBCH89 (Table 5.8).

TABLE 5.5 – F-statistics and significance levels from the performed three-way ANOVA.

Source of variation	**DM of the total aboveground biomass**		**N uptake of the total aboveground biomass**		**N content of the total aboveground biomass**		**Leaf/Stem ratio**		**LAR**	
	Df	F-value	Df	F-value	Df	F-value	Df	F-value	Df	F-value
Year (Y)	2	173***	2	122***	1	17***	1	1	1	3
Stage (S)	3	243***	3	51***	3	827***	3	88***	2	3612***
N fertilization (N)	3	17***	3	43***	6	26***	6	2	6	4**
Replicate (R)	3	1	3	1	3	4**	3	2	3	5*
Y*S	6	18***	6	6***	3	22***	3	3	2	27***
Y*N	6	1	6	1	6	3**	6	1	6	4**
S*N	9	2*	9	3***	18	5***	18	2*	12	13***
Y*S*N	18	1	18	1	18	2	18	2	12	5***

Note. '*' indicating statistical significance at p-value<0.05 ; '**' indicating statistical significance at p-value<0.01 ; '***' indicating statistical significance at p-value<0.001.

Table 5.6 – Leaf/Stem ratio of the aboveground biomass for the different N fertilizations and phenological stages from (A) 2020 to (B) 2021. Data are presented as average ± standard error.

		Leaves/stems ratio				
		BBCH30	***BBCH39***	***BBCH65***	***BBCH89***	***Mean of stages***
(A) 2020	*0+0+0N*	5.58±0.46	0.76±0.05	0.31±0.02	0.25±0.03	1.72±0.59
	50+0+0N	4.41±0.55	0.73±0.03	0.27±0.03	0.23±0.02	1.41±0.47
	50+0+50N	6.02±1.41	0.56±0.04	0.24±0.03	0.21±0.01	1.76±0.71
	100+0+0N	4.68±0.98	0.66±0.05	0.24±0.02	0.21±0.01	1.45±0.53
	100+50+0N	4.76±1.15	0.61±0.08	0.25±0.03	0.18±0.02	1.45±0.56
	0+100+0N	4.95±0.50	0.76±0.05	0.40±0.04	0.21±0.02	1.58±0.52
	50+50+50N	5.00±0.92	0.64±0.06	0.32±0.03	0.22±0.01	1.54±0.56
	Mean of fert.	5.06±0.32 A	0.67±0.02 B	0.29±0.01 B	0.22±0.01 B	
(B) 2021	*0+0+0N*	15.70±9.81 A	0.66±0.02a B	0.26±0.01 B	0.17±0.01 B	3.43±2.33
	50+0+0N	9.09±0.30 A	0.59±0.05ab B	0.24±0.01 B	0.16±0.01 B	2.08±0.94
	50+0+50N	7.64±3.44 A	0.50±0.03b B	0.21±0.01 B	0.15±0.01 B	2.13±1.13
	100+0+0N	4.49± 0.36 A	0.49±0.05b B	0.24±0.01 B	0.14±0.01 B	1.34±0.48
	100+50+0N	4.18±0.66 A	0.47±0.02b B	0.21±0.02 B	0.15±0.01 B	1.25±0.46
	0+100+0N	6.00± 0.99 A	0.57±0.03ab B	0.22±0.02 B	0.14±0.01 B	1.73±0.68
	50+50+50N	3.40±0.24 A	0.46±0.03b B	0.22±0.02 B	0.15±0.01 B	1.06±0.36
	Mean of fert.	6.82±1.29	0.53±0.02	0.23±0.01	0.15±0.01	

Note. Means with a letter differ significantly (p-value$<$0.05). Letters in minuscule represent the result of SNK test of the effect of fertilization and letters in majuscule represent the result of SNK test of the effect of phenological stage. When letters are reported in the 'mean of fert.' row or in the 'mean of stages' column, it means no interactions between those factors were reported for that year.

TABLE 5.7 – Leaf area ratio of the aboveground biomass for the different N fertilizations and phenological stages from (A) 2020 to (B) 2021. Data are presented as average ± standard error.

		Leaf area ratio				
		BBCH30	***BBCH39***	***BBCH65***	***BBCH89***	***Mean of stages***
(A) 2020	*0+0+0N*	0.0084±0.0009c A	0.0066±0.0008a B	0.0016±0.0003b C	0.0055±0.0031	0.0084±0.0009c A
	50+0+0N	0.0105±0.0008b A	0.0054±0.0003bc B	0.0016±0.0003b C	0.0058±0.0038	0.0105±0.0008b A
	50+0+50N	0.0133±0.0014a A	0.0051±0.0003bc B	0.0017±0.0002b C	0.0067±0.0051	0.0133±0.0014a A
	100+0+0N	0.0129±0.0012a A	0.0057±0.0006ab B	0.0016±0.0003b C	0.0068±0.0049	0.0129±0.0012a A
	100+50+0N	0.0129±0.0017a A	0.0042±0.0008c B	0.0020±0.0004b C	0.0064±0.0050	0.0129±0.0017a A
	0+100+0N	0.0103±0.0006b A	0.0052±0.0006bc B	0.0026±0.0001a C	0.0060±0.0034	0.0103±0.0006b A
	50+50+50N	0.0116±0.0007ab A	0.0050±0.0006bc B	0.0021±0.0001b C	0.0062±0.0042	0.0116±0.0007ab A
	Mean of fert.	0.0114±0.0020	0.0053±0.0009	0.0019±0.0004		0.0114±0.0020
(B) 2021	*0+0+0N*	0.0116±0.0012ab A	0.0056±0.0005 B	0.0006±0.0001b C	0.0060±0.0047	0.0116±0.0012ab A
	50+0+0N	0.0129±0.0008ab A	0.0051±0.0005 B	0.0010±0.0001a C	0.0064±0.0052	0.0129±0.0008ab A
	50+0+50N	0.0124±0.0008ab A	0.0051±0.0002 B	0.0008±0.0001ab C	0.0061±0.0050	0.0124±0.0008ab A
	100+0+0N	0.0123±0.0011ab A	0.0047±0.0007 B	0.0009±0.0001ab C	0.0060±0.0050	0.0123±0.0011ab A
	100+50+0N	0.0133±0.0009a A	0.0049±0.0006 B	0.0008±0.0003ab C	0.0063±0.0055	0.0133±0.0009a A
	0+100+0N	0.0112±0.0005b A	0.0055±0.0004 B	0.0011±0.0002a C	0.0060±0.0044	0.0112±0.0005b A
	50+50+50N	0.0117±0.0004ab A	0.0046±0.0005 B	0.0006±0.0001b C	0.0056±0.0048	0.0117±0.0004ab A
	Mean of fert.	0.0122±0.0010	0.0051±0.0006	0.0008±0.0002		0.0122±0.0010

Note. Means with a letter differ significantly (p-value$<$0.05). Letters in minuscule represent the result of SNK test of the effect of fertilization and letters in majuscule represent the result of SNK test of the effect of phenological stage. When letters are reported in the 'mean of fert.' row or in the 'mean of stages' column, it means no interactions between those factors were reported for that year.

TABLE 5.8 – Aboveground biomass production (t DM ha^{-1}) for the different N fertilizations and phenological stages in 2019, 2020 and 2021 for (A) leaves and stems and (B) ears. Data are presented as average ± standard error.

(A)		**DM of leaves and stems ± S.E. (t ha^{-1}))**				
		BBCH30	**BBCH39**	**BBCH65**	**BBCH89**	**Mean of stages**
2019	**0+0+0N**	1.8±0.1	7.8±1.5	12.2±1.2	13.8±2.2	8.9±1.4
	50+0+0N	2.1±0.2	11.3±1.5	11.7±0.5	13.4±1.4	9.5±1.3
	50+0+50N	2.2±0.2	13.7±3.6	16.6±1.5	14.3±0.5	11.6±1.7
	100+0+0N	2.2±0.2	15.6±1.9	13.5±0.7	13.5±0.7	10.6±1.5
	100+0+50N	2.7±0.5	12.6±3.4	14.0±1.0	14.2±0.7	10.7±1.5
	Mean of fert.	2.2±0.1	11.7±1.2	13.6±0.6	13.8±0.5	
		A	B	C	C	

		DM of leaves ± S.E. (t ha^{-1}))					**DM of stems ± S.E. (t ha^{-1}))**				
		BBCH30	**BBCH39**	**BBCH65**	**BBCH89**	**Mean of stages**	**BBCH30**	**BBCH39**	**BBCH65**	**BBCH89**	**Mean of stages**
2020	**0+0+0N**	0.9±0.1	1.5±0.3	1.2±0.1	0.9±0.2	1.1±0.1c	0.2±0.0	1.9±0.3	3.9±0.1	3.5±0.7	2.4±0.4c
	50+0+0N	1.2±0.1	2.0±0.1	1.3±0.1	1.2±0.1	1.4±0.1ab	0.3±0.1	2.7±0.2	4.8±0.4	5.6±1.0	3.3±0.6abc
	50+0+50N	1.5±0.2	2.1±0.3	1.2±0.1	1.0±0.1	1.5±0.1ab	0.3±0.1	3.8±0.7	5.6±1.1	4.8±0.5	3.6±0.6ab
	100+0+0N	1.4±0.2	2.3±0.2	1.4±0.1	1.2±0.1	1.6±0.1a	0.4±0.1	3.5±0.2	5.8±0.9	5.7±0.6	3.8±0.6ab
	100+50+0N	1.5±0.2	1.9±0.3	1.5±0.1	1.2±0.2	1.5±0.1a	0.4±0.2	3.5±0.9	6.5±0.9	6.8±0.7	4.3±0.7a
	0+100+0N	1.2±0.1	1.5±0.1	1.3±0.1	0.9±0.1	1.2±0.1bc	0.3±0.1	2.0±0.1	3.6±0.6	4.4±0.4	2.5±0.4c
	50+50+50N	1.1±0.1	1.9±0.1	1.4±0.1	0.9±0.1	1.3±0.1abc	0.3±0.1	3.1±0.4	4.5±0.7	3.9± 0.2	3.0±0.5bc
	Mean of fert.	1.3±0.1	1.9±0.1	1.3±0.1	1.1±0.1		0.3±0.0	2.9±0.2	4.9±0.3	5.0±0.3	
		B	A	B	C		A	B	C	C	
2021	**0+0+0N**	0.7±0.1d A	1.2±0.1c B	1.2±0.1b B	1.0±0.1 AB	1.0±0.1	0.1±0.0d A	1.8±0.2d B	4.5±0.4b C	5.80±0.3b D	3.1±0.6
	50+0+0N	0.9±0.1cd A	1.6±0.1bc B	1.5±0.2ab B	1.1±0.1 A	1.3±0.1	0.1±0.0d A	2.7±0.2cd B	6.2±0.8ab C	6.9±0.7ab C	4.0±0.8
	50+0+50N	1.4±0.3abc	1.8±0.1bc	1.6±0.1ab	1.2±0.1	1.5±0.1	0.3±0.1bc A	3.6±0.2bc B	7.7±0.5a C	7.7±0.5ab C	4.8±0.8
	100+0+0N	1.5±0.1abc AB	1.9±0.1b B	2.0±0.2a B	1.2±0.1 A	1.7±0.1	0.3±0.0bc A	4.0±0.4bc B	8.7±1.0a C	8.1±0.8ab C	5.3±0.9
	100+50+0N	1.7±0.1ab A	2.7±0.3a B	1.6±0.2ab A	1.1±0.1 A	1.8±0.2	0.5±0.1ab A	5.9±0.7a B	7.8±1.2a B	7.9±0.6ab B	5.5±0.9
	0+100+0N	1.1±0.2bcd A	1.7±0.2bc B	1.4±0.2ab AB	1.2±0.0 A	1.4±0.1	0.2±0.1cd A	3.1±0.3cd B	6.4±0.5ab C	8.4±0.4ab D	4.5±0.8
	50+50+50N	2.0±0.1a A	2.2±0.2b A	1.6±0.1ab B	1.3±0.1 B	1.8±0.1	0.6±0.1a A	4.7±0.6ab B	7.1±0.2ab C	9.0±0.9a D	5.4±0.8
	Mean of fert.	1.3±0.1	1.9±0.1	1.6±0.1	1.2±0.1		0.3±0.1	3.7±0.3	6.9±0.4	7.7±0.3	

Table 5.8 (continued)

(B)		**DM of ears ± S.E. (t ha^{-1}))**		
		BBCH65	**BBCH89**	**Mean of stages**
2019	***0+0+0N***	1.6±0.2	2.6±0.4	2.1±0.3
	50+0+0N	1.5±0.1	2.3±0.2	1.9±0.2
	50+0+50N	2.1±0.3	2.2±0.5	2.2±0.3
	100+0+0N	1.6±0.2	2.0±0.4	1.8±0.2
	100+0+50N	1.6±0.4	1.9±0.6	1.7±0.4
	Mean of fert.	1.7±0.1 A	2.2±0.2 B	
2020	***0+0+0N***	0.5±0.1	0.6±0.1	0.5±0.1c
	50+0+0N	0.6±0.1	1.2±0.4	0.9±0.2bc
	50+0+50N	0.9±0.1	1.2±0.1	1.1±0.1b
	100+0+0N	1.0±0.2	1.5±0.2	1.2±0.2b
	100+50+0N	1.2±0.2	2.1±0.3	1.6±0.2a
	0+100+0N	0.5±0.1	1.1±0.1	0.8±0.1bc
	50+50+50N	0.8±0.2	1.0±0.1	0.9±0.1bc
	Mean of fert.	0.8±0.1 A	1.2±0.1 B	
2021	***0+0+0N***	0.7±0.0	1.1±0.1	0.9±0.1b
	50+0+0N	1.0±0.1	1.4±0.2	1.2±0.1ab
	50+0+50N	1.3±0.1	1.5±0.2	1.4±0.1a
	100+0+0N	1.4±0.2	1.6±0.3	1.5±0.2a
	100+50+0N	1.3±0.2	1.7±0.2	1.5±0.2a
	0+100+0N	1.2±0.2	1.8±0.2	1.5±0.2a
	50+50+50N	1.4±0.1	1.9±0.3	1.6±0.2a
	Mean of fert.	1.2±0.1 A	1.6±0.1 B	

Note. Means with a letter differ significantly (p-value$<$0.05). Letters in minuscule represent the result of SNK test of the effect of fertilization and letters in majuscule represent the result of SNK test of the effect of phenological stage. When letters are reported in the ‘mean of fert.’ row or in the ‘mean of stages’ column, it means no interactions between those factors were reported for that year and plant organ.

The comparison between biomass production levels of the different aerial organs indicates a higher amount of leaves than stems at BBCH30. At BBCH39 and after, stems are the most represented organ of the plant. At BBCH30 ($\sum$UPVT of 191), leaves represented 83.8 ± 2.5 % of the total aboveground biomass. Reversely, they accounted for 12.9 ± 0.7 % at BBCH89, while stems and ears represented respectively 71.4 ± 1.4 % and 15.7 ± 1 % of the total aboveground biomass (Figure 5.4). The leaf/stem ratio seemed to be only influenced by phenological stages. The ratio was found to decrease during the growing season (Table 5.6). The same trend was observed for the leaf area ratio, as it was significantly influenced by phenological stages ($p\text{-value}<0.001$), with a sharp decrease from BBCH30 to BBCH65 (Table 5.7).

N fertilization had generally a positive impact on the aboveground DM production, especially on vegetative organs. Indeed, in 2020, the lowest biomass of leaves and stems were obtained with the reference treatment (0+0+0N) and the high mid-spring fertilization (0+100+0N), regardless of the stage of development. In 2021, the biomass from both stems and leaves was also the lowest with the reference treatment, the high mid-spring fertilization and the low early-spring fertilization (50+0+0N) at early stages of the crop cycle (BBCH30 and BBCH39). Later in the growing season, the biomass of vegetative organs remained broadly equivalent for all N treatments, except in the reference treatment which has always the lowest level of biomass production. Focusing on the biomass of ears, the 100+50+0N treatment showed the highest level in 2020. But apart from this situation, the biomass of ears was not significantly influenced by the different N treatments, with no difference compared to the reference treatment in 2019, and only lower levels of biomass found for the reference treatment in 2021 (Table 5.8).

3.1.2. Evolution and partitioning of plant tissues N content

Significant interactions were found between the fixed factors (Table 5.5). Detailed results within each year and each plant organ (when available) are illustrated in Figure 5.5, for the treatment 3, and presented in (Table 5.9).

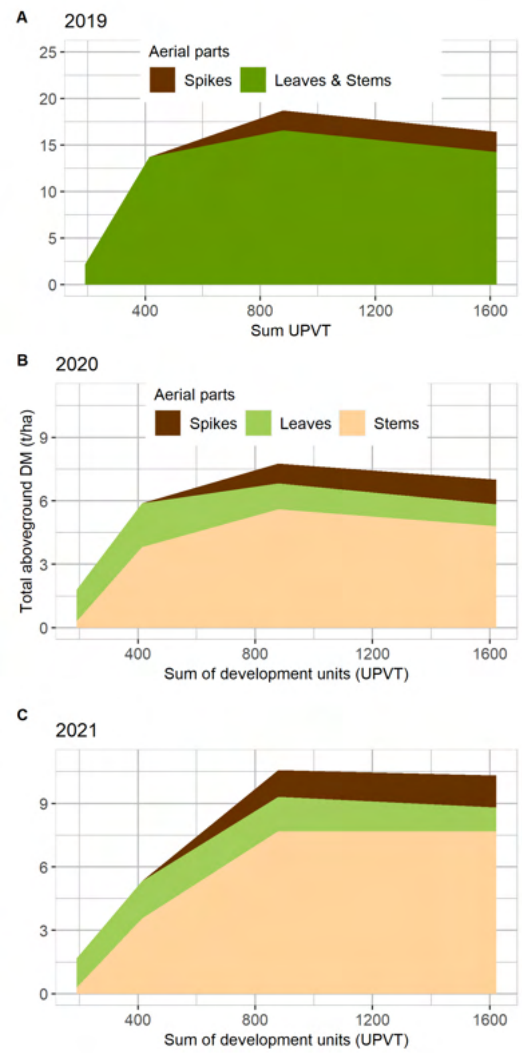

FIGURE 5.4 – Aboveground DM (t ha^{-1}) partitioning in plant organs according to the accumulation of crop UPVT in (A) 2019, (B) 2020 and (C) 2021 for the N treatment 3 (50+0+50N).

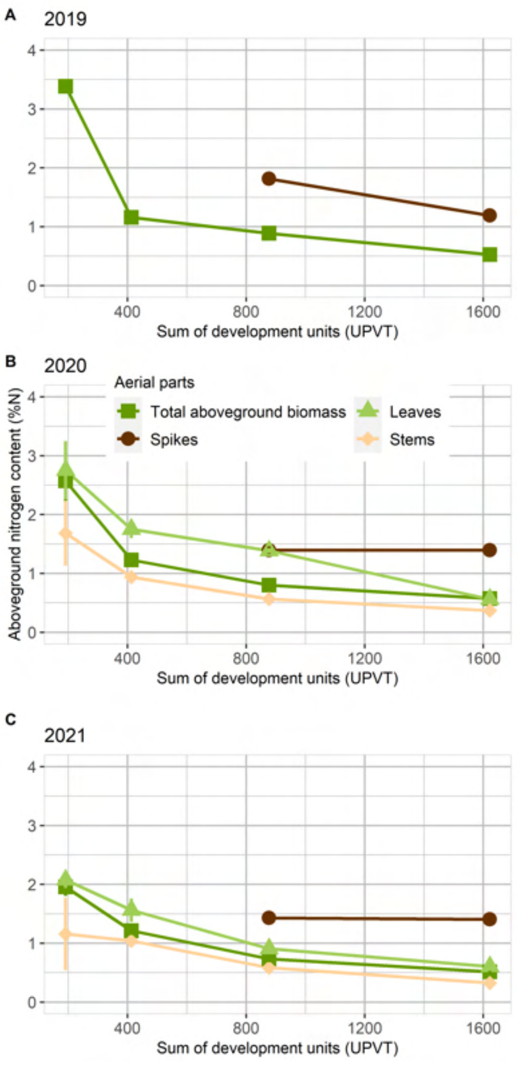

FIGURE 5.5 – Aboveground N content (%N) of plant organs according to the accumulation of crop UPVT in (A) 2019, (B) 2020 and (C) 2021 for the N treatment 3 (50+0+50N).

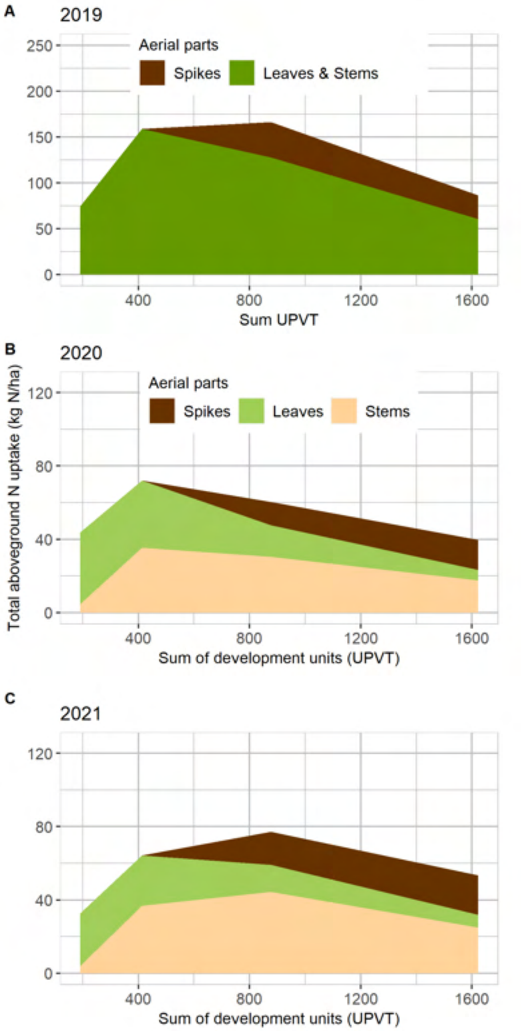

FIGURE 5.6 – Aboveground N uptake (kg N ha^{-1}) partitioning in plant organs according to the accumulation of crop UPVT in (A) 2019, (B) 2020 and (C) 2021 for the N treatment 3 (50+0+50N).

TABLE 5.9 – N content (%N) within the aboveground biomass for the different N fertilizations and phenological stages for in 2019, 2020 and 2021 for (A) leaves and stems and (B) ears. Data are presented as average ± standard error. In 2019 at the BBCH89 stage no statistical analyses were performed, as the N content was measured on averaged samples.

(A)		N content of leaves and stems ± S.E. (%)									
2019		**BBCH30**	**BBCH39**	**BBCH65**	**BBCH89**	**Mean of stages**					
	0+0+0N	2.40±0.04c	0.91±0.01b	0.50±0.02b	0.34	1.04					
	50+0+0N	2.90±0.01b	1.08±0.08b	0.56±0.03b	0.32	1.22					
	50+0+50N	3.23±0.05a	0.99±0.06b	0.54±0.08b	0.43	1.30					
	100+0+0N	3.35±0.07a	1.16±0.08ab	0.72±0.08a	0.37	1.40					
	100+0+50N	3.46±0.03a	1.32±0.04a	0.80±0.03a	0.35	1.48					
	Mean of fert.	3.09±0.08	1.09±0.04	0.62±0.03	0.36						
		N content of leaves ± S.E. (%)					**N content of stems ± S.E. (%)**				
2020		**BBCH30**	**BBCH39**	**BBCH65**	**BBCH89**	**Mean of stages**	**BBCH30**	**BBCH39**	**BBCH65**	**BBCH89**	**Mean of stages**
	0+0+0N	2.13±0.09c A	1.09±0.10b B	0.90±0.05e B	0.46±0.01d C	1.14±0.16	1.19±0.05bc A	0.78±0.09a B	0.38±0.06c C	0.32±0.02b C	0.67±0.09
	50+0+0N	2.40±0.13bc A	1.34±0.11b B	1.18±0.05d B	0.48±0.01d C	1.35±0.18	1.39±0.09abc A	0.80±0.04a B	0.48±0.04bc C	0.31±0.01b D	0.74±0.11
	50+0+50N	2.75±0.25ab A	1.75±0.08a B	1.38±0.02c B	0.56±0.02cd C	1.61±0.21	1.68±0.28ab A	0.94±0.04a B	0.57±0.03b BC	0.37±0.01b C	0.89±0.14
	100+0+0N	3.01 ±0.12a A	2.19±0.13a B	1.61±0.02b C	0.65±0.04bc D	1.86±0.23	1.74±0.12ab A	1.11±0.11a B	0.68±0.04a C	0.37±0.02b D	0.98±0.14
	100+50+0N	3.26 ±0.18a A	2.15±0.15a B	1.86±0.10a C	0.81±0.06a D	2.02±0.23	1.92±0.24a A	1.12±0.12a B	0.80±0.04a BC	0.49±0.02a C	1.09±0.15
	0+100+0N	2.02 ±0.06c A	1.25±0.03b C	1.83±0.03a B	0.73±0.04ab D	1.46±0.13	1.11±0.08c A	0.76±0.06a B	0.78±0.05a B	0.48±0.03a C	0.78±0.06
	50+50+50N	2.98 ±0.27a A	1.91±0.19a B	1.75±0.04ab B	0.72±0.03ab C	1.84±0.22	1.73±0.20ab A	1.00±0.11a B	0.76±0.03a BC	0.46±0.02a C	0.99±0.13
	Mean of fert.	2.65±0.10	1.67±0.09	1.50±0.07	0.63±0.03		1.54±0.08	0.93±0.04	0.64±0.03	0.40±0.02	
2021											
	0+0+0N	1.81±0.07b A	1.31±0.03c B	1.01±0.15b C	0.53±0.02c D	1.17±0.13	1.10±0.18	1.15±0.10	0.51±0.01	0.34±0.01	0.77±0.10c
	50+0+0N	2.09±0.05b A	1.48±0.02bc B	0.94±0.04b C	0.53±0.01c D	1.26±0.15	1.19±0.20	1.07±0.04	0.57±0.01	0.33±0.02	0.79±0.10c
	50+0+50N	2.07±0.07b A	1.56±0.10abc B	0.90±0.02b C	0.60±0.04bc D	1.29±0.15	1.16±0.31	1.04±0.05	0.58±0.02	0.32±0.04	0.78±0.11c
	100+0+0N	2.47±0.22a A	1.73±0.05ab B	0.99±0.06b C	0.66±0.03bc C	1.46±0.19	1.71±0.12	1.05±0.02	0.61±0.03	0.33±0.03	0.92±0.14abc
	100+50+0N	2.51±0.08a A	1.94±0.07a B	1.30±0.12b C	0.90±0.07a D	1.66±0.16	1.69±0.06	1.26±0.18	0.94±0.04	0.45±0.05	1.08±0.13a
	0+100+0N	1.77±0.13b A	1.85±0.17ab A	1.21±0.18b B	0.70±0.06bc C	1.38±0.14	1.20±0.12	1.16±0.05	0.79±0.14	0.35±0.03	0.88±0.10bc
	50+50+50N	2.16±0.16b A	1.72±0.11ab A	1.78±0.27a A	0.79±0.07ab B	1.61±0.15	1.47±0.02	1.31±0.17	0.91±0.08	0.41±0.06	1.03±0.11ab
	Mean of fert.	2.13±0.07	1.66±0.05	1.16±0.07	0.67±0.03		1.36±0.07 A	1.15±0.04 B	0.70±0.04 C	0.36±0.02 D	

Table 5.9 (continued)

(B)		**N content of ears ± S.E. (%)**		
2019		**BBCH65**	**BBCH89**	**Mean of stages**
	0+0+0N	1.71±0.04	1.13	1.42
	50+0+0N	1.89±0.09	1.15	1.52
	50+0+50N	1.95±0.05	1.19	1.57
	100+0+0N	1.96±0.02	1.10	1.53
	100+0+50N	1.89±0.03	1.19	1.54
	Mean of fert.	1.88±0.03	1.15	
2020	***0+0+0N***	1.37±0.04	1.30±0.11	1.33±0.06b
	50+0+0N	1.43±0.08	1.32±0.03	1.37±0.05b
	50+0+50N	1.39±0.03	1.39±0.03	1.39±0.02b
	100+0+0N	1.47±0.04	1.46±0.04	1.46±0.03b
	100+50+0N	1.57±0.08	1.73±0.05	1.65±0.05a
	0+100+0N	1.65±0.04	1.72±0.06	1.69±0.03a
	50+50+50N	1.61±0.07	1.55±0.06	1.58±0.04a
	Mean of fert.	1.50±0.03	1.50±0.04	
2021	***0+0+0N***	1.34±0.11	1.37±0.07	1.35±0.06b
	50+0+0N	1.33±0.03	1.40±0.04	1.37±0.02b
	50+0+50N	1.43±0.06	1.41±0.05	1.42±0.04ab
	100+0+0N	1.60±0.05	1.47±0.04	1.53±0.04ab
	100+50+0N	1.76±0.16	1.57±0.11	1.66±0.10a
	0+100+0N	1.61±0.20	1.49±0.11	1.55±0.11ab
	50+50+50N	1.84±0.12	1.56±0.05	1.70±0.08a
	Mean of fert.	1.56±0.05	1.46±0.03	

Note. Means with a letter differ significantly ($p\text{-value} < 0.05$). Letters in minuscule represent the result of SNK test of the effect of fertilization and letters in majuscule represent the result of SNK test of the effect of phenological stage. When letters are reported in the ‘mean of fert.’ row or in the ‘mean of stages’ column, it means no interactions between those factors were reported for that year and plant organ.

Overall, the N content of vegetative organs (leaves and stems) decreased along the crop cycle. As illustrated in Figure 5.5, the highest N content of leaves and stems was obtained at BBCH30 ($\sum$UPVT of 191). Reversely, the phenological stage of the crop had no significant influence on the N content of ears which was similar between BBCH65 ($\sum$UPVT of 878) and BBCH89 ($\sum$UPVT of 1622) stage (Figure 5.5).

As expected, at each stage of crop development, the N content was higher in leaves than in stems. At BBCH89, the N content was the lowest in stems and the highest in ears (Figure 5.5).

The N content in aboveground organs increased with the N fertilization. Globally the absence of autumn or early-spring fertilization lowered N content in vegetative organs at BBCH30 while the absence of mid-spring fertilization lowered N content of leaves and stems at BBCH65 and BBCH89. Concerning the N content of ears, the SNK's results showed a globally higher N content with the mid-spring fertilization by increasing it by 0.3 % compared to the reference treatment (Table 5.9).

3.1.3. Evolution and partitioning of N uptake in the aboveground biomass

Significant interactions were reported between the fixed factors (Table 5.5). Therefore, Table 5.10 presents detailed results within each year and each plant organ, when available. Overall, the N uptake of the total aboveground biomass increased from the BBCH30 to BBCH39 ($\sum$UPVT of 413) or BBCH65 ($\sum$UPVT of 878) stages before decreasing until BBCH89 as shown in Figure 5.6 for treatment 3. The N uptake decrease from leaves is generally more pronounced than in stems, which tend to accumulate N later and conserve it longer (Table 5.10, Figure 5.6). Looking at ears, the N uptake generally increased between BBCH65 and BBCH89. However, this increase in ears does not compensate the N uptake decrease in vegetative organs in late growing season, resulting in total N uptake diminution in the aboveground biomass (Table 5.10).

The N uptake of the aboveground biomass tended to increase with the N fertilization. The lowest N uptake of leaves and stems was always obtained with the reference treatment. At the beginning of the growing season (BBCH30), the N uptake of vegetative organs is increased by high early spring fertilization (100+0+0N and 100+50+0N treatments) and by early spring fertilization coupled with autumn fertilization (50+0+50N and 50+50+50N treatments). At BBCH89, the influence of fertilization seemed more limited although the lowest N uptake of leaves and stems is obtained with the reference treatment

(Table 5.10). The N fertilization had no influence on the N uptake by ears in 2019. The reference treatment and the 50+0+0N fertilization seemed to limit N uptake by ears in 2020 and 2021 while the highest N uptake of ears was obtained with the 100+50+0N fertilization in 2020 and with the 50+50+50N fertilization in 2021 (Table 5.10). The limited impact of the N fertilization on the N uptake of the total aboveground biomass at BBCH89 was also reflected by the ANR efficiencies, that were low and variable between the growing seasons and the N fertilization treatments as observed in Table 5.11.

3.2. Establishment of the CNDC (BE1 and V1-5 sites)

Coefficients of the CNDC of *Th. intermedium* with their 95 % credibility interval are presented in Table 5.12 and their posterior densities are graphically represented in Figure 5.7.

The newly established CNDC is presented in Figure 5.8 and Figure 5.9A. It highlights that the N content decreases with the aerial biomass production of *Th. intermedium*. Regarding the dataset used to validate the results (V1-5 sites), the newly developed CNDC seemed to properly separate the 'limiting' and 'non-limiting' N situations, while some minor errors remained. The expert-based information surimposed to some data might have led to situations where points are not fully N stressed, especially during the early crop growth where they can benefit from residual soil N and early mineralization. Yet, the discrimination of 'limiting' and 'non-limiting' N situations remains globally very efficient.

Figure 5.9B shows the newly developed CNDC of *Th. intermedium* compared with other crops presented in Table 5.1. With a much lower *a*-coefficient, the CNDC of *Th. intermedium* appeared to be positioned under the curves of *Triticum aestivum* (a C3 annual species) and *Zea mays* (a C4 annual species) obtained by the Bayesian method (Table 5.1 and Table 5.12). The closest crops in terms of behavior appeared to be *Miscanthus giganteus* & *sinensis* (Figure 5.9B) or Grapevine (Table 5.1).

TABLE 5.10 – Aboveground N uptake (kg N ha^{-1}) for the different N fertilizations and phenological stages in 2019, 2020 and 2021 for (A) leaves and stems and (B) ears. Data are presented as average ± standard error.

(A)		N uptake of leaves and stems ± S.E. (kg N ha^{-1}))									
		BBCH30	**BBCH39**	**BBCH65**	**BBCH89**	**Mean of stages**					
2019	***0+0+0N***	46.0±1.7b	73.0±13.6	67.5±6.6b	46.9±7.4	58.4±4.9					
	50+0+0N	61.6±6.5ab A	148.0±19.4 B	73.7±2.9b A	43.5±4.4 A	77.3±10.6					
	50+0+50N	74.4±6.5ab A	159.0±41.5 B	127.9±11.4a B	60.6±2.2 A	101.9±12.9					
	100+0+0N	78.9±7.2ab A	218.2±25.9 C	127.6±7.0a B	49.5±2.7 A	104.3±15.8					
	100+0+50N	91.7±16.1a AB	149.6±39.9 B	98.5±7.0a AB	49.4±2.4 B	93.8±12.1					
	Mean of fert.	70.5±5.0	139.9±16.5	99.0±6.6	50.0±2.2						
		N uptake of leaves ± S.E. (kg Nha^{-1}))					**N uptake of stems ± S.E. (kg Nha^{-1}))**				
		BBCH30	**BBCH39**	**BBCH65**	**BBCH89**	**Mean of stages**	**BBCH30**	**BBCH39**	**BBCH65**	**BBCH89**	**Mean of stages**
2020	***0+0+0N***	19.7±3.0c A	16.5±4.1c AB	10.6±1.1d BC	4.0±0.8c C	12.7±1.9	2.0±0.2 A	14.2±1.0c B	14.5±1.8c B	11.0±1.9b B	10.4±1.5
	50+0+0N	29.2±3.6bc A	26.2±2.0bc A	14.9±1.0cd B	5.9±0.6bc C	19.0±2.6	4.0±0.7 A	21.5±0.2bc B	22. 5±1.3bc B	17.5±3.5b B	16.4±2.1
	50+0+50N	39.3±3.1ab A	36.6±5.5b A	17.1±1.3bc B	5.7±0.5bc C	24.7±3.9	4.6±1.2 A	35.4±5.6a C	30.5±4.2bc C	17.6±1.2b B	22.0±3.5
	100+0+0N	41.6±5.8ab A	51.2±7.1a A	21.8±1.5ab B	7.6±0.7b B	30.6±4.9	5.9±1.5 A	39.1±4.3a C	39.4±5.4ab C	20.9±1.4b B	26.3±3.9
	100+50+0N	47.9±7.0a A	41.4±6.8ab AB	28.2±2.5a B	9.9±1.2a C	31.8±4.4	8.1±3.0 A	36.6±6.5a B	51.9±7.2a C	33.3±3.9a B	32.5±4.7
	0+100+0N	23.8±1.8bc A	18.4±0.9c B	24.4±1.6a A	6.7±0.5bc C	18.3±1.9	2.9±0.6 A	14.9±1.5c B	28.1±5.8bc C	20.5±0.6b BC	16.6±2.7
	50+50+50N	30.9±1.2abc A	35.5±1.9b A	24.2±2.4a B	6.2±0.5bc C	24.2±3.0	4.0±0.8 A	30.0±3.3ab C	34.8±6.3b C	18.2±1.5b B	21.7±3.5
	Mean of fert.	33.2±2.3	32.3±2.7	20.2±1.3	6.6±0.4		4.5±0.6	27.4±2.3	31.7±2.7	19.9±1.4	
2021	***0+0+0N***	12.8±1.1b A	15.5±1.9d A	12.1±2.6b A	5.3±0.7b B	11.4±1.2	0.9±0.5c B	20.3±2.5b A	22.7±1.6c A	19.7±0.9b A	15.9±2.4
	50+0+0N	19.3±2.5b A	23.3±0.9cd A	14.3±2.0b B	5.9±0.5b C	15.7±1.8	1.1±0.4c C	29.0±2.0b AB	35.4±4.3bc A	22.2±1.5ab B	21.9±3.5
	50+0+50N	28.6±5.4ab A	27.5±0.8bc A	14.7±0.7b B	7.0±0.8ab B	19.4±2.6	4.2±1.8bc C	36.9±1.4b A	44.5±1.4abc A	25.0±3.9ab B	27.6±4.1
	100+0+0N	37.9±6.9a A	33.0±0.4bc AB	20.2±2.7ab BC	7.7±1.0ab C	24.7±3.5	5.7±0.5ab D	41.5±3.1b B	53.2±5.1abc A	26.7±3.3ab C	31.8±4.8
	100+50+0N	42.3±3.2a A	52.9±5.2a A	20.4±1.6ab B	10.4±1.5a B	31.0±4.0	7.4±1.4ab B	72.8±9.6a A	72.9±11.5a A	35.5±4.6a B	47.2±7.9
	0+100+0N	18.3±2.3b B	32.4±4.6bc A	17.7±3.8ab B	8.1±0.7ab C	19.1±2.7	2.3±0.7c C	35.6±3.2b AB	51.1±10.1abc A	29.5±2.6ab B	29.6±5.2
	50+50+50N	43.3±3.5a A	36.6±2.5b A	27.0±3.0a B	10.4±0.9a C	29.3±3.4	8.9±1.0a C	61.4±9.5a A	64.7±7.2ab A	35.7±1.9a B	42.7±6.4
	Mean of fert.	28.9±2.6	31.6±2.3	18.1±1.2	7.8±0.5		4.4±0.7	42.5±3.8	49.2±3.8	27.8±1.5	

Table 5.10 (continued)

(B)		**N uptake of ears ± S.E. (kg N ha^{-1}))**		
		BBCH65	**BBCH89**	**Mean of stages**
2019	***0+0+0N***	27.6±3.0	29.1±4.6	28.4±2.5
	50+0+0N	26.5±1.0	25.9±1.7	26.2±0.9
	50+0+50N	38.6±4.6	25.9±5.5	32.2±4.1
	100+0+0N	31.2±4.5	21.9±4.8	26.5±3.5
	100+0+50N	27.9±7.8	22.5±7.5	25.2±5.1
	Mean of fert.	30.3±2.1	25.1±2.1	
2020	***0+0+0N***	6.7±1.0	7.4±0.6	7.0±0.6c
	50+0+0N	8.4±1.2	15.9±4.7	12.2±2.7bc
	50+0+50N	12.9±1.5	16.3±1.4	14.6±1.1b
	100+0+0N	14.7±3.2	21.0±3.1	17.9± 2.4b
	100+50+0N	18.7±2.9	35.4±5.3	27.1±4.2a
	0+100+0N	8.8±2.2	18.9±1.5	13.8±2.3b
	50+50+50N	12.6±3.0	15.7±1.2	14.1±1.1b
	Mean of fert.	11.8±1.1 A	18.7±1.8 B	
2021	***0+0+0N***	9.4±0.8	15.3±1.9	12.4±1.5c
	50+0+0N	13.5±1.7	19.1±3.5	16.3±2.1bc
	50+0+50N	18.0±1.0	21.5±3.5	19.7±1.8abc
	100+0+0N	21.9±3.1	23.7±4.3	22.8±2.5ab
	100+50+0N	21.8±2.9	26.8±4.3	24.3±2.6ab
	0+100+0N	20.3±4.8	26.5±2.8	23.4±2.8ab
	50+50+50N	25.9±3.1	28.7±4.2	27.3±2.5a
	Mean of fert.	18.7±1.4 A	23.1±1.5 B	

Note. Means with a letter differ significantly ($p\text{-value} < 0.05$). Letters in minuscule represent the result of SNK test of the effect of fertilization and letters in majuscule represent the result of SNK test of the effect of phenological stage. When letters are reported in the 'mean of fert.' row or in the 'mean of stages' column, it means no interactions between those factors were reported for that year and plant organ.

TABLE 5.11 – ANR efficiency and NUE for the different N fertilization treatments and growing seasons.

	ANR			Aboveground NUE		
	2019	2020	2021	2019	2020	2021
0+0+0N						
50+0+0N	-0.1 ± 0.2	0.2 ± 0.1	0.1 ± 0.2	1.4 ± 0.2	0.6 ± 0.1	0.9 ± 0.2
50+0+50N	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.8 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
100+0+0N	-0.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.2	0.7 ± 0.1	0.5 ± 0.1	0.6 ± 0.2
100+0+50N	-0.03 ± 0.1			0.5 ± 0.1		
100+50+0N		0.4 ± 0.1	0.2 ± 0.1		0.5 ± 0.1	0.5 ± 0.1
0+100+0N		0.2 ± 0.1	0.2 ± 0.1		0.5 ± 0.1	0.6 ± 0.1
50+50+50N		0.1 ± 0.1	0.2 ± 0.1		0.3 ± 0.1	0.5 ± 0.1

TABLE 5.12 – Coefficients of the proposed CNDC of *Th. intermedium* and their 95 % credibility interval.

	***a*-coefficient**	***b*-coefficient**	**$\%N = aMS^{-b}$**
CNDC	2.35	0.46	$\%N = 2.35M^{-0.46}$
95 % credibility interval	[1.25 ; 4.10]	[0.19 ; 0.76]	

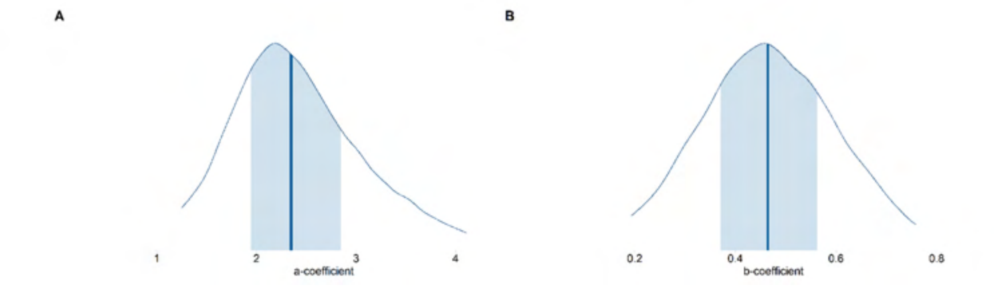

FIGURE 5.7 – Posterior densities of (A) *a*- and (B) *b*-coefficient of the CNDC. Distributions are presented over the range of their 95 % credibility interval. Dark blue lines represent the median value and light blue zones represent the 50 % credibility interval.

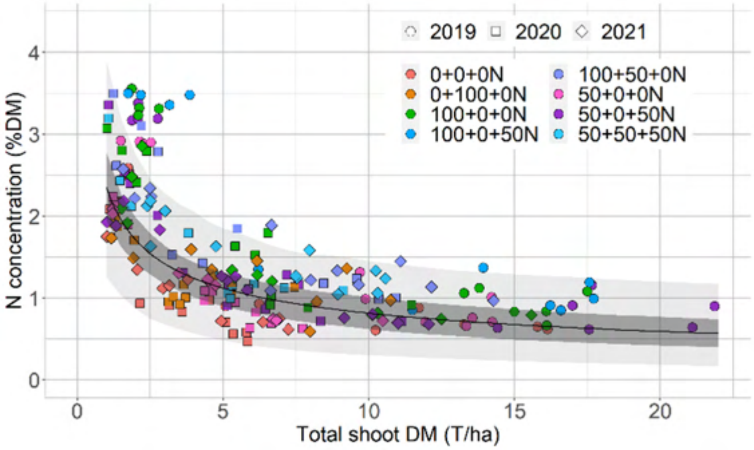

FIGURE 5.8 – Relationship between N concentration of the total aboveground biomass (%) and DM production of *Th. intermedium* from the Belgian experimental BE1 dataset used to set up the CNDC. Black line represent the CNDC generated with median parameter value, dark grey area represented its 50% CI and light grey area its 95% CI of CNDC.

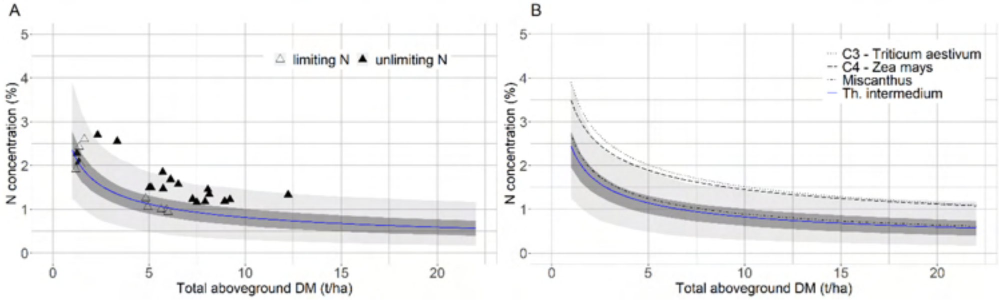

FIGURE 5.9 – (A) Newly developed CNDC of *Th. intermedium* set up over the BE1 dataset and plotted against the V1-5 validation dataset. (B) Comparison of the CNDC of *Th. intermedium*, *Tritcum aestivum* (C3 annual plant), *Zea mays* (C4 annual plant) and *Miscanthus* (C4 perennial plant). Blue line represent the CNDC generated with median parameter value, dark grey area represented the 50 % CI and light grey area the 95 % CI of CNDC of *Th. intermedium*.

4. Discussion

4.1. Area of validity of the CNDC

Our proposed CNDC was established through hierarchical Bayesian modelling, as proposed by Makowski et al. (2020). This statistical method offers the possibility to estimate the CNDC without classifying limiting and non-limiting N data and without assuming that W_{max} has been reached in all sampling dates. In addition, the uncertainty in the fitted CNDC is analyzed concomitantly (Fernandez et al., 2022). As shown in Figure 5.9A, the proposed CNDC seemed to properly separate the 'limiting' and 'non-limiting' N situations from the dataset used to validate the curve. However, as explained by Fernandez et al. (2022), to obtain an accurate and precise CNDC with this method, the use of a minimum of eight experiments is recommended. In this study, only six field experiments could be used due to the lack of data on this developing crop species. In the future, the dataset could be expanded to further validate this initial CNDC. In addition, the impact of biomass sampling at ground level compared to 5 cm above the soil surface could be assessed.

4.2. Understanding N needs through the CNDC

Globally, the maximization of the aboveground production of DM and the N uptake was obtained with a N application comprised between 100 and 150 kg ha^{-1} applied over the entire growing year (including autumn fertilization). However, depending upon the year and the phenological stage, the increase might not always be significant compared to reduced fertilizations strategies (i.e., 50 kg N ha^{-1} over the entire growing year). Particularly, low ANR efficiencies (i.e., always under 40 %) were observed at grain harvest, that indicated a poor response of *Th. intermedium* to N fertilization (Table 5.11). Compared to the reference N treatment, fertilized treatments only absorbed a small proportion of the N applied in the aboveground biomass at the end of the growing season. Nevertheless, N fertilization should be controlled to optimize the final yields, as observed by Jungers et al. (2017). In their study, they found the optimum N fertilization to range between 61 and 96 kg N ha^{-1} applied in spring to maximize yields with an average of 10.8 t DM ha^{-1} at the grain maturity stage. Using our proposed N dilution curve, the N content for this level of biomass production is averaging 0.79 % corresponding to a N uptake of 85 kg ha^{-1}. If we consider our maximizing N treatments (application ranging between 100 and 150 kg ha^{-1}), the Belgian mean biomass yield at grain maturity stage was around 11.5 t DM ha^{-1} over the three cropping seasons. It corresponded to an average N content of 0.76 % resulting in a N uptake of 88 kg ha^{-1}. Production levels are relatively similar, corresponding to comparable N requirements that should be met through N supply from the soil and/or the fertilization. Thus, regarding these relatively low N needs, N fertilizations should be adjusted to the yield targets, the different soil and climatic conditions of the field including soil N availability to be at the optimum. In addition, to further optimize N fertilization, we recommend studying the response of the crop to finer fertilization amount intervals. Yet, the newly proposed CNDC provides a robust characterization of the N critical status and needs for *Thinopyrum intermedium*, as a response to crop management practices or environmental conditions (Lemaire et al., 2019). However, as *Th. intermedium* has a recent selection history, the potential development of new genotypes or cultivars should be studied in the future as it could lead to different growing patterns, therefore influencing N requirements or the CNDC (Lemaire et al., 2019).

Concerning the N fertilization timings of *Th. intermedium*, a late summer or autumn fertilization could be integrated into the N management strategy of the multi-annual *Th. intermedium* crop. Indeed, a autumn N application combined with an early-spring application resulted in relatively similar aboveground production levels of the crop as a full early-spring N application if we compare treatment 3 and 4 of our study. In addition, as Cattani and Asselin (2017) highlighted, N applied in autumn could enhance fertile tiller initiation and N applied in spring during pre-reproductive induction could allow a better N use by inducing larger fertile tillers, larger panicles, and greater seed set. Therefore, spring applications could be reduced by transferring a part of these applications in late summer or autumn or in very early-spring before reproductive induction. This strategy could also prevent risks of lodging at the end of the growing cycle, lodging that has been observed in the study of Jungers et al. (2017) under high spring N fertilizations.

During the crop cycle of *Th. intermedium*, N is diluted in the aboveground biomass, which results in a reduction of the N percentage. Furthermore, data reported in this study indicated that the LAR of *Th. intermedium* decreased along the growing season. As explained in the study of Ratjen et al. (2018), as aboveground biomass increases, growth becomes more vertical and leaves are organized in leaf-layers which progressively differentiate leaf declination, specific leaf area and vertical N distribution. The fraction of structural biomass which has a low N concentration is known to increase at a higher relative rate than the metabolic fraction which is characterized by a high N concentration. In that way, the metabolic fraction varies with the photosynthetic surface of the plant while the structural fraction varies with the canopy height and leaf thickness. Therefore, it has been shown that, in a large range of crops, the decline of N percentage is strictly parallel to the decline of the leaf area ratio (Ratjen et al., 2018; Lemaire and Belanger, 2020). Our results provided evidences that the dilution of N within *Th. intermedium* tissues might respond to the same mechanisms.

4.3. Storing nutrients in perennial structures to ensure survival strategy

After an initial increase until BBCH39 or BBCH65, the N uptake within the aboveground biomass was found to decrease during the second part of the growing season as shown in Figure 5.6. For some treatments, we observed a decrease of up to 50 % of the N uptake within the aboveground biomass during the second phase of the growing season. Surprisingly, N fertilization appeared to not really influence this N disappearance, not allocated to grains. Neither was the addition of N as fertilizer associated to any substantial increase in the allocation of N towards the grains. While it has no impact in 2019, the fertilization of 150 kg N ha^{-1} increased the N uptake of ears by only around 20 kg in 2020 and 12 kg in 2021 in comparison to the reference treatment. Allocation of N to ears seems much lower compared to other cultivated crops such as wheat (Hussain et al., 2006). A first explanation lies in the low allocation of DM to grains. We observed that ears, including grains and vegetative biomass such as seed hulls and rachis, represented approximatively 16 % of the aboveground biomass at grain harvest for the treatment 3. A study conducted by Culman et al. (2013) reported that grains represented only 10 % of total aboveground DM of *Th. intermedium*, compared to 50 % for annual winter wheat that allocates much more resources to seeds.

We believe that the low seeds production and the associated low N uptake in the grains are related to a long-term survival strategy of the plant, which can translocate nutrients to belowground organs at the expense of grains production. This has been previously underlined in the study of Nassi o Di Nasso et al. (2013) for *Arundo Donax* L., a C3 rhizomatous grass. The plant experienced a peak nutrients level in shoots over the summer period followed by a decline and a simultaneous increase in belowground rhizomes' level. At the end of the growing season, the crop exhibited relatively low nutrient contents in shoots. The same trend was reported for C4 crops (*Miscanthus* & *Spartina cynosuroides*), which translocates nutrients to rhizomes at the end of each growing season, with a mean N content within the aboveground biomass declining respectively by 83 % and by 77 % for *M. x giganteus* and *S. cynosuroides* (Beale and Long, 1997). The latter species produce larger quantities of rhizomes than

Th. intermedium, approximately 50 % of the belowground biomass for *Arundo donax* (Quinn et al., 2007) and between 60 to 80 % at shallow depth considering different *Miscanthus* species and growth years (Dohleman et al., 2012; Christensen et al., 2016) compared to 17 % for *Th. intermedium* (Sakiroglu et al., 2020). As reported in the study of Sakiroglu et al. (2020), nutrients are stored in rhizomes of *Th. intermedium* suggesting that this organ could play an important role in spring regrowth and plant survival. In addition, in this latter study, it was hypothesized that the storage of reserves in rhizomes for spring regrowth would be significant in the first few years and would then decrease with the age of the crop. Another belowground storage organ could be represented by the root system, where N might be stored and then remobilized to the shoot after defoliation to support leaf regrowth. This was observed for alfalfa or ryegrass. The N reserves stored in alfalfa roots and that contributed to shoot regrowth reached 30 kg N ha^{-1} in the study of Lemaire et al. (1992). Finally, ground-level stem's bases might be another storage organ used by *Th. intermedium* in its survival strategy. As proposed in the review of White (1973) about perennial grasses, we also believe that the lower region of the stems (i.e., the stem's bases) could also be a storage area of most carbohydrate reserves for *Th. intermedium* that could be used as an energy source to initiate new growth following herbage removal.

Considering all these aspects, it is not unlikely that *Th. intermedium* would have similar internal mechanisms for the reallocation of nutrients toward belowground organs (i.e., roots and short rhizomes) or ground-level organs (i.e., stem's bases). That was effectively observed in the previous chapter (Figure 4.10) with the increase of the N uptake within stem bases after flowering. During regrowth, *Th. intermedium* could use the nutrients stored in these organs to develop plants already established (from reserves of roots and/or stem bases) or produce the shoot and root systems of new plants (from rhizomes - as hypothesized by Sakiroglu et al. (2020).

4.4. Linking N use efficiency with resource-conservative strategy

The newly proposed CNDC of *Th. intermedium* seemed very different from other annual crop species' CNDC, such as *Triticum aestivum* L. or *Zea mays* L. (Figure 5.9B), as well as from other perennial grasses' CNDC (Table 5.1). *Th. intermedium* had globally much lower needs in terms of N nutrition translated in a *a*-coefficient of 2.35. Based on the estimations of *a*- and *b*-coefficients, it seems that the N amount needed for intermediate wheatgrass would be approximatively 60 % of the N needed by *Triticum aestivum* at a production of 1 t DM ha^{-1}, and 53 % at a production of 15 t DM ha^{-1}. These differences in N use efficiency - mostly related to a lower a-coefficient (Lemaire and Salette, 1981) - have been highlighted in the pioneer work of Greenwood et al. (1990) who identified clear differences between C3 and C4 metabolic groups. The lower a-coefficient reported for *Th. intermedium* can be associated to aerial tissues with lower N content. At low levels of W (1 t DM ha^{-1}), aboveground biomass is mainly composed of leaves ; this would reflect a lower leaves' N content. For treatment 3, the leaves' N content was about 2.8 % in 2020 and 2.1 % in 2021 at the beginning of the growing season for a biomass of 1.5 and 1.3 t DM ha^{-1} respectively.

In addition, we found out that the CNDC of *Th. intermedium* is relatively close from the one reported by Zapater et al. (2017) for *Miscanthus* (Figure 5.9 - B). In this study, such low N needs have been related to several life traits. Potential explanation lies in the work of Beale and Long (1997) who reported (i) a higher NUE (ii) a high nutrient uptake efficiency thanks to a deep and extensive root system and (iii) an efficient nutrient recycling through translocation from shoots to rhizomes and through remobilization from rhizomes to shoots the following growing season. Our calculated aboveground NUE were similar to the study of Sprunger et al. (2018a) (Table 5.11). In this study, they also considered the simplest whole-plant NUE definition as the N content of the whole plant (roots included) divided by the N applied. The whole-plant NUE of *Th. intermedium* was very high, and the plant seemed to be able to assimilate large quantities of N, even greater than what was applied. This same study reported that *Th. intermedium* allocated between 23 to 50 % of biomass to roots. Its deep and dense root system allows an extensive exploration of the soil profile which can further increase the NUE while at the same time reducing nitrate leaching (Jungers et al., 2019).

Beyond these aspects, the higher NUE of *Th. intermedium* could be discussed in link with different growing habits and with a resource-conservative strategy of the crop. As explained by the theory of Tilman (1982), in low soil fertility conditions, the rate of acquisition of nutrients would be low and plants would grow very slowly. The plants having the more efficient uptake capacity for the more limiting resource, and/or the ability to store and to conserve this resource through efficient internal recycling mechanisms will be more competitive. The concept of 'resource conservation' within the plant has thus been highlighted : 'as the time of residence of one resource within a plant increase, this resource becomes more efficient and in consequence it can be acquired in lower quantity for maintaining the plant alive' (Lemaire, 2001). Therefore, species with long leaf life span should have a lower demand for N resources and should persist better in a poor soil condition than species with short leaf life span. The ability for acquiring and conserving resources, for most herbaceous plant species, can be described by leaf traits (i.e., specific leaf area, DM content of leaf, leaf N % and leaf life span), allowing a rapid classification between slow- and fast-growing species (Lemaire, 2001). In the study of Maire et al. (2009) this N conservative strategy has been related to different physiological traits. Indeed, some tall grass species with high N-yields (i.e., N uptake of shoots) and high root and shoot biomass can display more conservative traits such as a high leaf N use efficiency combined with a low leaf N concentration and a low root uptake capacity which is the case of Dactylis glomerata or Festuca arundinacea. Furthermore, Duchene et al. (2020) hypothesized that some root traits of *Th. intermedium* were also linked to a resource-conservative strategy of the plant, namely the higher tissue density, suggesting an enhanced root storage functions with a higher residence time of nutrients in tissues.

5. Conclusions

Our study has highlighted that *Th. intermedium* perennial grain crop is able to reach a high shoot DM production with low N needs. This is most likely associated to its long-term survival strategy that implies an important investment in perennial structures coupled with a weaker resource allocation to reproductive seeds. Some growing patterns of the crop were put in relation with mechanisms observed in plant with similar strategies, such as the N recycling through the storage of nutrients in the perennial organs or an extensive exploration of soil with a dense and deep root system allowing for a certain efficiency at extracting nutrients. In this study we didn’t evaluate the N contents of roots, rhizomes and stem bases of *Th. intermedium* to confirm the possible translocation of nutrients to these belowground or ground-level organs during the second part of the growing season. However, in the previous chapter an increase of the amount of N within stem bases was observed after flowering. Overall, the CNDC found in this study will be highly helpful to help define N requirements in various pedo-climatic environments and adjust accordingly the soil-crop management, and more precisely the management of the N fertilization. Ultimately, the low N requirements of *Th. intermedium* coupled with a high NUE demonstrate that the crop can enhance agronomic and environnemental benefits such as (i) the N cycling and accumulation in soil by its belowground and/or storage organs, (ii) the reduction of nitrate leaching or (iii) the potential to produce high aboveground biomass in N limited environnements.

6

Caractérisation de l’allocation des ressources de *Thinopyrum intermedium* vers le grain

Introduction au chapitre

Ce chapitre est adapté d'une publication dans le journal 'European Journal of Agronomy' :

`https://doi.org/10.1016/j.eja.2023.127038`

Suite à une meilleure compréhension de l'écologie fonctionelle et des besoins azotés de la culture, ce chapitre se concentre sur l'élaboration du rendement en grains de *Th. intermedium*. Pour ce faire, de multiples variables caractérisant le développement de la culture ont été suivies sur quatre années au champ. Les mécanismes conduisant à l'optimisation du rendement en grains de la culture au champ ont été identifiés. Divers leviers agronomiques ont été mis en évidence pour optimiser ces rendements dans le temps, dont notamment la fertilisation azotée.

Maintaining grain yield of *Thinopyrum 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 ; +3281622141

[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

Credit authorship contribution statement

L.F. : conceptualization, planning of the experiments, formulation of research goals and aims, development and design of methodology, carrying out the samplings, data curation, formal analyses (statistical and mathematical), interpretation of result, data presentation and visualization, writing – original draft preparation.

B.D. : supervision.

F.C., O.D., B.D. : help provided for data presentation, contributed to the interpretation of result, critical review, commentary and revision, validation.

Keywords

Perennial grain crop, Nitrogen fertilization, Autumn defoliation, Constant grain yield, Kernza, *Thinopyrum intermedium*.

Abstract

Thinopyrum 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 au-

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

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

The understanding of grain yield build-up of *Th. intermedium* is still limited, but recent studies identified multiple positive correlations between the seed yield and several traits such as aboveground biomass, reproductive tiller density, spike length, plant height or flag leaf area (Zhang et al., 2016; Cattani and Asselin, 2018; DeHaan et al., 2018; Bajgain et al., 2019; Bajgain and Anderson, 2021). Altendorf et al. (2021) suggests that biomass per se is a poor indicator of grain yield, while the reproductive tiller density at harvest and floret site utilization (ranging from 12 to 60 %, Larson et al. (2019); Altendorf et al. (2021)) are the main drivers in spaced plants. However, in denser swards, a strong trade-off has been observed between tiller density and fertility, especially as stand ages (Jungers et al., 2017; Hunter et al., 2020a), so that total densities exceeding roughly 1000 tillers m^{-2} were detrimental to grain yield. Additionally, there is a marked trend of yield decline as stand ages, leading to a drop in the harvest index as high as 50 % (Culman et al., 2023; Duchene et al., 2023). Different productivity dynamics have been observed in previous studies, with yields peaking the first or the second year of cultivation before dropping down (Jungers et al., 2017; Tautges et al., 2018; Bajgain et al., 2020; Bergquist et al., 2022). These various patterns may be explained by compensatory effects between yield per spike (i.e., grain weight and number) and yield per plant (driven by reproductive tiller number) (Altendorf et al., 2021). Several processes have been hypothesized to explain yield decline, including increased competition for light or nutrients with higher tiller density (Jungers et al., 2017; Tautges et al., 2018), more stress-tolerant growth strategy (Law et al., 2020), or resource allocation shifts toward perennating organs (Vico et al., 2016). At the present time, the selection process does not detect a strong opposition between the increase in grain yield and the vigor of regrowth. On the contrary, grain yield improvement has been suggested to be feasible without major loss of perennation through extended growing season and enhanced resource availability and use (Vico et al., 2016; DeHaan et al., 2018).

Beside breeding, Bajgain et al. (2022) estimated that 31 % of global yield increase would be linked to better management in fields, as it was seen for wheat. Among this, N management is one major factor. N availability influences crop yield mostly through its effect upon canopy expansion, survival, and longevity of organs (Hay and Porter, 2006; Satorre and Slafer, 1999). The effect on tiller development, survival and growth in perennial grasses has long been demonstrated (Gislum and Griffith, 2004; Assuero and Tognetti, 2010). When seeking to maximizing grain production, N shortage in autumn or early-spring can be detrimental to yields due to source-limited conditions for tiller development, growth, and spikelet's formation (Langer, 1980; Boelt and Studer, 2010; Reynolds et al., 2022). However, the effect of N fertilization for seed-bearing grasses is controversial due to unclear effect on tiller and spike fertility, increased lodging risk or secondary tillering (Langer, 1980; Boelt and Studer, 2010). Some studies have investigated the impact of N fertilization on *Th. intermedium* performance (Jungers et al., 2017; Frahm et al., 2018; Fagnant et al., 2023) and suggested an optimal N fertilization ranging from 61 to 100 kg N ha^{-1}. Defoliation operations can have a major impact on grass productivity as well. Defoliation performed above the apex height is known to preserve the tiller reproductive potential. Meanwhile, defoliation may generate new vegetative tillers and could eventually delay stem elongation or reduce tiller height (Mansat and Pfitzenmeyer, 1966; Gillet, 1973; Rouet et al., 2021). With respect to *Th. intermedium*, various management options have been studied to improve grain yield in young stands or to maintain yield in aging stands including crop defoliation in summer (i.e., just after grain harvest), in autumn or in spring, either by grazing (Dick et al., 2018) or mowing. As the spring mowing tends to decrease grain yield (Zimbric et al., 2021; Culman et al., 2023), the summer and autumn mowing can increase grain yield compared to no forage harvesting (Pugliese et al., 2019; Culman et al., 2023), but without mitigating yield decline with stand age (Hunter et al., 2020a).

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

2. Materials and methods

2.1. Experimental design

A field experiment (BE1) was conducted in the experimental farm of ULiège – Gembloux Agro-Bio Tech, Belgium (50°33’58.7” N, 4°42’22.2” E), during four cropping years. A split-plot randomized complete block design was used, with defoliation as the main-plot treatment and N fertilization as the split-plot treatment, with four replicates of each treatment. Defoliation factor compared two treatments : i) no autumn defoliation and ii) autumn defoliation. During autumn mechanical defoliation vegetation was clipped to a height of 7 cm and removed simultaneously. Defoliation was only performed in the autumn (i.e., early November) of the first and third cropping year due to insufficient plant regrowth the other years (Table 6.1). The N fertilization treatments (ammonium nitrate granular) differed according to the total amount and the timing of application and were modified during the study with only some N treatments present during the four cropping years (Table 6.1). Each subplot (i.e., split plot of 4 * 8 m) was divided by two to obtain one plot being dedicated to grain yield measurement in summer and the other one to destructive sampling during the growing season. The experiment was sown on September 22^{nd}, 2017, with an interrow spacing of 25 cm and a density of 250 seeds m^{-2} leading to a plant density of 176 plants m^{-2} in the first cropping year. Seeds used originate from the fifth breeding cycle achieved at The Land Institute (Kansas, USA). The field is characterized by deep and fertile soil conditions with a clay-loam soil type, and optimal nutrients content with a phosphorus content averaging 0.16 mg g^{-1}, potassium content averaging 0.22 mg g^{-1} and organic carbon content averaging 13 g kg^{-1} over the four years of the experiment. Chemical weeding (i.e., 80 g ha^{-1} of clopyralid, 120 g ha^{-1} of fluoxypyr and 800 g ha^{-1} of MCPA on November 17^{th} , 2017, coupled with 960 l ha^{-1} of bentazon and 30 g ha^{-1} of amidosulfuron on April 18th, 2018) and two mechanical weeding (i.e., interrow hoe weeder in late May 2018) were only performed during the first cropping year. Most post-harvest residues (i.e., straw) were removed manually from the field and the remaining part was shredded at 5 cm from the ground after each grain harvest.

TABLE 6.1 – (A) N fertilization treatments described with their timings and amounts (kg ha^{-1}) and (B) defoliation treatments of the experimental site during the four cropping years and the statistical analyses performed on the different treatments.

Management treatments					**Cropping year where treatment was studied**				**Stat. ana-lyses**
(A) N fertilization									
		Splitting (kg N ha^{-1})							
Treatment number	**Total N dose (kg N ha^{-1})**	**Early-spring***	**Mid-spring***	**Autumn vegetative stage**	**2018**	**2019**	**2020**	**2021**	
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 ana
Summer straw harvest (no autumn defoliation)					x	x	x	x	

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

2.2. Data collection

Data were collected during the four cropping years of the experiment (representing four grain production years with a first establishment year and three successive regrowing years). Aboveground biomass was sampled with a 50 x 50 cm quadrat per plot, cut at 5 cm above soil surface, oven-dried (72 h at 60 °C) and weighted to obtain DM. Samples were collected at five different main phenological stages during the growing season ; four reproductive stages rated with the BBCH scale (Meier, 2018), namely the stem elongation (BBCH30), the flag leaf (BBCH39), the flowering (BBCH65) and the grain maturity (BBCH89) stages as well as a vegetative stage during the autumn regrowth, as illustrated in Figure 4.7. Spikes, when present, were always separated from the remaining aboveground biomass (i.e., cut just below the lowest spikelet) to be weighted. When collecting total aboveground biomass, the number of tillers and spikes during the reproductive phase (i.e., from BBCH30 to BBCH89 stage) were counted within the quadrat and tiller fertility were 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 stage by the number of tillers at BBCH30 stage.

At grain harvest, plots were combined with a trial combine harvester to obtain the grain yield on a DM basis and cleaned, but unsorted seeds basis (i.e., a mix of hulled and dehulled seeds). Few days before grain harvest, plant height was measured randomly by selecting five plants from each plot and measuring them from the ground to the end of the spike. On the same five random plants, the length of the spike was simultaneously measured. Thousand kernel weight was determined on each plot using four replicates by counting 100 seeds with a seed counter and then weighting them. Thousand kernel weight (TKW) was measured on a bare seed basis as well as on unsorted seeds (TKW_{un}). TKW_{un} was then used to calculate spike fertility and grain density using Equations (6.1) and (6.2) (Hay and Porter, 2006).

$$\textit{spike fertility} = \frac{\textit{grain yield}}{\textit{spike density}.\frac{TKW_{un}}{1000}} \tag{6.1}$$

$$\textit{grain density} = \textit{spike fertility.spike density} \tag{6.2}$$

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

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

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

2.3. Weather conditions

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

During the vegetative and reproductive phases 5 main indicators were calculated : 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 as performed in Duchene et al. (2021) and finally the water balance (Ks coefficient). Water balances were drawn up to assess soil water availability and to estimate the daily depletion of the extractable soil water (total available water (TAW)) to a depth of 2 m as performed by Duchene (2020). The water balance was calculated from January 31, 2017 (considering a complete filling of the TAW) to estimate the soil water status at the beginning of the establishment year. The daily evolution of the TAW until the end of the experiment was calculated and then determined as a proportion of the total readily available water to calculate the stress indicator (Ks). Equations are presented in the work of Duchene (2020). The various weather indicators are presented in Table 6.2. Unfortunately, the variability within these weather indicators was too low to integrate them within the different data analyses (i.e., correlation analysis and path analysis). The response of crop measurements to these indicators was too weak.

2.4. Data analyses

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

2.4.1. Standard statistical analysis

N fertilizations were modified during the study, with only some treatments present in all four cropping years (i.e., treatment 1, 2, 3 and 4) and the defoliation was only performed in the autumn of 2018 and 2020 (Table 6.1). Data subsets were used to performed ANOVA as described in Table 6.1 and Table 6.3. Within the different ANOVA conducted, mixed models were used with *lmer* function from the *lme4* package. A two-way ANOVA was first performed where fixed factors were constituted of the N fertilization and the defoliation. A second two-way ANOVA was performed on the autumn non-defoliated treatments, with the N fertilization and stand age as fixed factors. As tiller density and total DM were measured at different phenological stages during the different cropping years a third fixed factor (i.e., the phenological stage) was added on these two mixed models. When an interaction was observed between factors, ANOVA were performed on the different treatments of the various fixed factors. Within the different mixed models, block, main plot and year, when year was not included as fixed effect through stand age, were considered as random effects. Models were evaluated to ensure they met the assumptions of independence and normality of residuals through the *plotresid* function. Following ANOVA analysis, pairwise comparisons among treatment means were evaluated with the *emmeans* function from the *emmeans* package with a Tukey adjustment for multiple comparisons. Statistical significance was set at 0.05. Grain yield, spike density, tiller fertility, TKW, spike fertility, grain density, spike length, plant height, N content in grains, harvest index, N harvest index, spike harvest index, fruiting efficiency, tiller density at BBCH30 and BBCH89, tiller mortality, spike DM at BBCH65 and BBCH89, lodging

TABLE 6.2 – Weather indicators of the experimental site during the four cropping years 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.

		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

and total DM from BBCH30 to BBCH89 and at the autumn regrowth were the analyzed variables.

2.4.2. Correlation analysis

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

2.4.3. Path analysis

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

2.4.3.1. Development of the initial model

The initial model described in Figure 6.1 was designed with the assumption and understanding that yield components develop sequentially, and that their effects on one another are unidirectional (i.e., represented by the sense of the arrow ; Figure 6.1), meaning that later developing components do not influence earlier developing components. Vegetative and maturity components are considered as exogeneous variables with no paths leading to them (Figure 6.1).

The initial model was constructed based on the available literature on *Th. intermedium* and on other grasses and grain crops. Regarding the yield per spike, spike fertility is shown as a function of spike length (Figure 6.1). Although highly variable, spike length of *Th. intermedium* was shown to be positively correlated with grain yield (Bajgain et al., 2019). TKW is a function of grain density (Figure 6.1), negative relationship between grain weight and grain density have been observed in previous studies on wheat (Slafer et al., 2022). Negative correlations were found between seed size and spikelets per spike or spikelet density in *Th. intermedium* (Bajgain et al., 2019; Altendorf et al., 2021). Concerning vegetative components (i.e., total DM, tiller density and plant height), they are defined to influence grain yield and its components (Figure 6.1). Indeed, the stem can be at play for translocation to grains during seed fill has shown in perennial ryegrass (Altendorf et al., 2021). In addition, plant height and total DM were shown to be positively correlated with grain yield in *Th. intermedium* (Zhang et al., 2016). Alternatively, grain yield decline in *Th. intermedium* stands over time could be due to the increase of tiller density that causes intraspecific competition and reduces seed production (Jungers et al., 2017) with a negative influence of tiller density and total DM on spike fertility (Hunter et al., 2020a). Finally, in wheat, grain density was related to the amount of resources allocated to the growing spikes (Altendorf et al., 2021; Slafer et al., 2022).

2.4.3.2. Model fitting

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

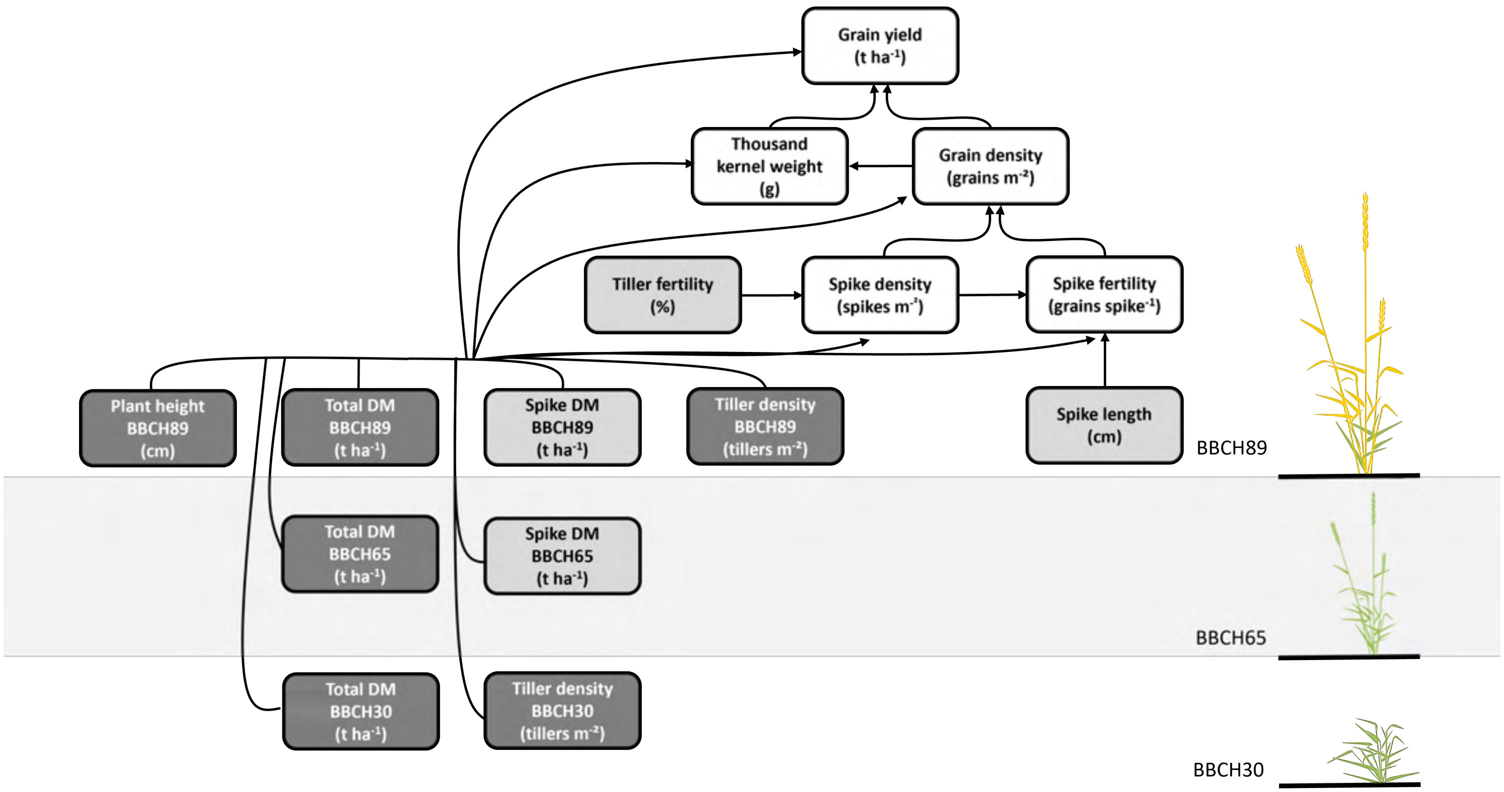

FIGURE 6.1 – Initial structural equation model for the relationship between grain yield and crop measurements, followed by their units in parentheses. Groups of traits are colored in different shades of gray, including grain yield and its components (in white), maturity traits (in medium gray) and vegetative traits (in dark gray). A direct path is represented by a single arrow that directly connects two traits (e.g., grain yield and grain density), whereas an indirect path is when the path between a pair of traits is separated by another trait (e.g., grain yield and spike density).

3. Results

3.1. Aboveground development

3.1.1. Impact of stand age and phenological stage

As shown in Figure 6.2, total aboveground biomass varied considerably across stand age (Table 6.3B). We observed the highest level of total DM during the second reproductive phase (i.e., 2019), reaching a mean of 15.8 ± 0.6 t of DM ha^{-1} at grain maturity stage (i.e., BBCH89). The three other reproductive phases (i.e., 2018, 2020 and 2021) presented similar levels of total DM at BBCH89 stage ranging from 5.7 ± 1.2 for the N-control treatment (i.e., treatment 1) to 10.5 ± 1.5 t of DM ha^{-1} for the N-sufficient treatment (i.e., treatment 4 that is sufficient to achieve a N non-limited crop development see Fagnant et al., (2023)). Total DM produced during the autumn regrowth was low and also significantly influenced by the stand age (Table 6.3B), with the highest level observed after the first grain harvest (e.g., mean of 1.0 ± 0.1 t of DM ha^{-1} in 2018 compared to 0.2 ± 0.1 t ha^{-1} the other years for the N-control treatment ; Figure 6.2).

Tiller density during the different reproductive phases is presented in Figure 6.3. Tiller density at the beginning of stem elongation (i.e., BBCH30) varied significantly across stand age (Table 6.3B), with the lowest level observed during the first year (i.e., 2018, with 463 ± 52 tillers m^{-2} on average) and the highest during the second year (i.e., 2019, with 1415 ± 48 tillers m^{-2} on average). By contrast, tiller density at BBCH89 stage was globally not influenced by the stand age (Table 6.3B). An exception can be mentioned with the N-control treatment where the highest tiller density at BBCH89 stage was observed in 2019 (p-value<0.001) due to particularly low tiller mortality (i.e., the decrease of tiller density during the reproductive phase ; Figure 6.3). Tiller mortality was the highest during the second year and the lowest during the first year. As opposed to the other years, tiller mortality was not observed in the first year as tiller density increased from BBCH30 to BBCH89 stages (Figure 6.3 ; Table 6.3B).

Plant height was significantly impacted by stand age (Table 6.3B). The tallest plants were observed in the second year (i.e., 2019 with a mean of 173 ± 2 cm) and the lowest were observed in 2020 (i.e., 125 ± 2 cm on average), marginal means are presented in Table 6.4.

TABLE 6.3 – 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.

	(A) Two-way ANOVA			(B) Two-way ANOVA		
Variables	**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				*	***	
N harvest index	/				***	
Lodging		***		***		**

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

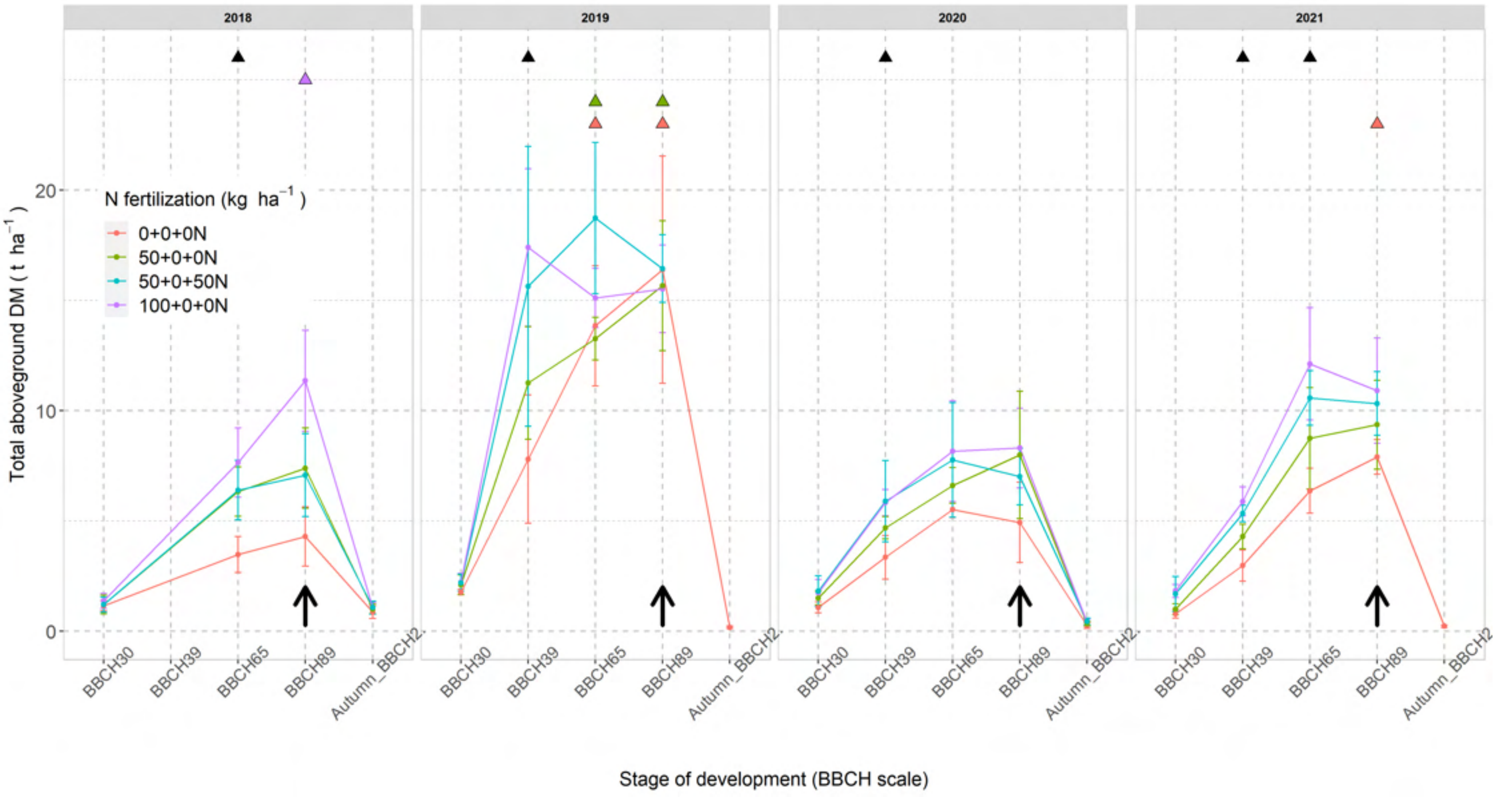

FIGURE 6.2 – Mean dynamic of total aboveground biomass during the growing season from 2018 to 2021 on the autumn non-defoliated treatment. Standard errors are represented by error bars. Statistical differences (p-value$<$0.05 ; post hoc analysis) between phenological stages are indicated by △ specifying an increased value ; and ▽ specifying a decreased value compared to the previous phenological stage. The color of the symbols represents the N treatment concerned and symbols in black represents the same trends for all N treatments. Arrows is specifying the time of grain harvest with the exportation of straw.

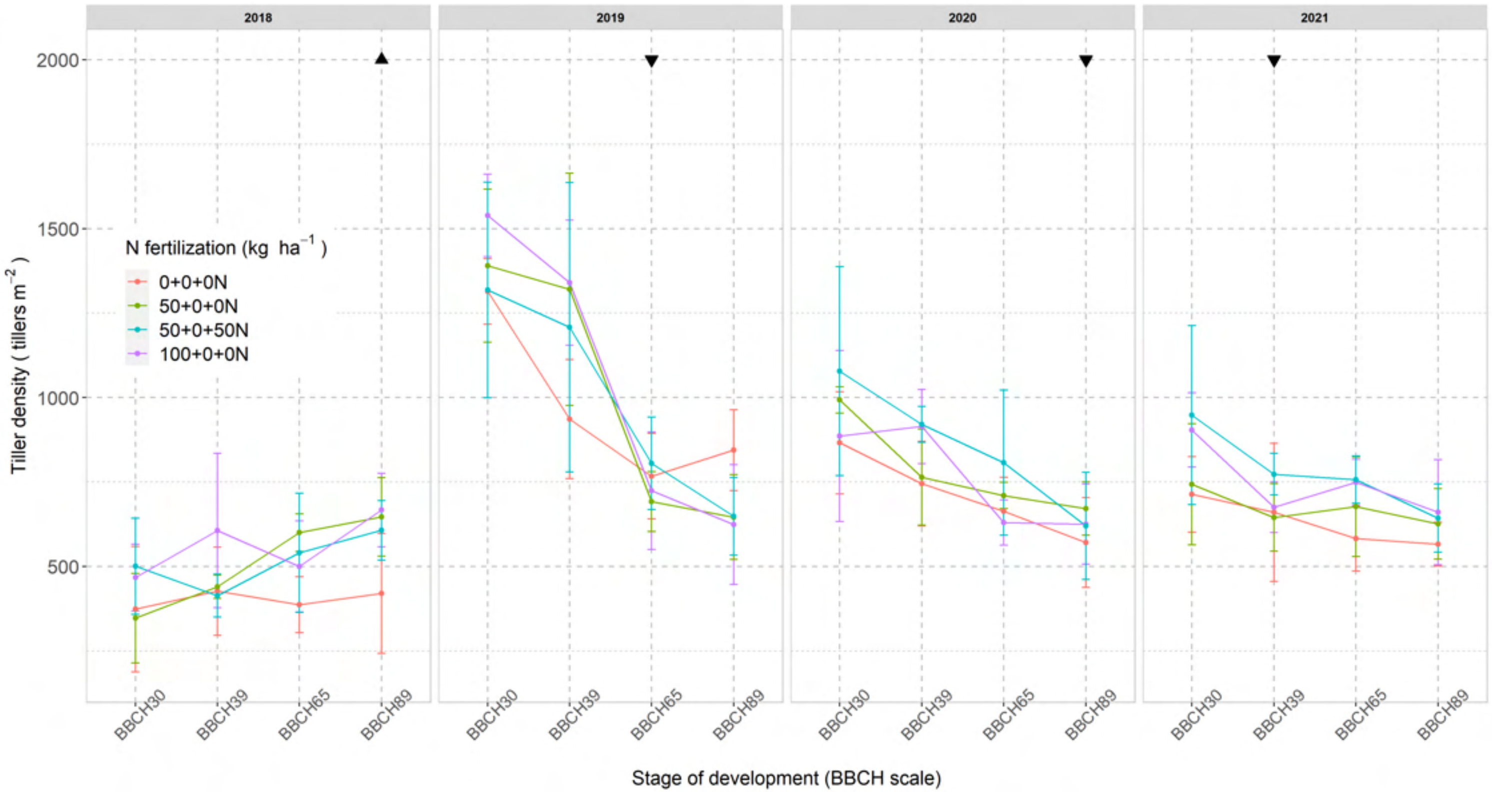

FIGURE 6.3 – Mean dynamic of tiller density during the reproductive phase from 2018 to 2021 on the autumn non-defoliated treatment. Standard errors are represented by error bars. Statistical differences (p-value$<$0.05 ; post hoc analysis) between phenological stages are indicated by △ specifying an increased value ; and ▽ specifying a decreased value compared to the previous phenological stage. The color of the symbols represents the N treatment concerned and symbols in black represents the same trends for all N treatments.

Table 6.4 – Marginal means ± standards errors of some crop measurements from 2018 to 2021 on the four common autumn non-defoliated N treatments. '/' is indicating that the variable was not measured on this treatment. Letters represent the results of the post hoc analysis of the effect of (A) N fertilization or (B) stand age.

	(A) N fertilization				**(B) Stand age**			
	0+0+0N	**50+0+0N**	**50+0+50N**	**100+0+0N**	**2018**	**2019**	**2020**	**2021**
Plant height (cm)	146±2b	152±2ab	156±2a	158±2a	149±2c	173±2a	125±2d	164±2b
Spike length (cm)	19±1	21±1	22±1	22±2	22±1ab	24±1a	19±1b	20±1b
Spike DM BBCH65 (t DM ha^{-1})	0.8±0.1c	1.0±0.1bc	1.3±0.1a	1.2±0.1ab	0.8±0.1c	1.7±0.1a	0.8±0.1c	1.1±0.1b
Spike DM BBCH89 (t DM ha^{-1})	1.3±0.1b	1.6±0.1ab	1.5±0.1ab	1.9±0.1a	1.5±0.2b	2.2±0.1a	1.1±0.1b	1.5±0.1b
Tiller fertility (%)	59±4b	73±4a	73±4a	72±4a	70±4a	77±4a	56±4b	74±4a
Harvest index (%)	11±1a	10±1ab	11±1ab	8±1b	12±1a	6±1b	11±1a	10±1a
N 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

3.1.2. Impact of N fertilization

Total aboveground DM varied considerably across N fertilizations (Table 6.3 - B). Globally, the N-control treatment had always the lowest total DM and moderate early-spring fertilization (i.e., treatment 2) appeared inadequate to increase total DM during the reproductive phase contrary to treatment 3 and 4 that had the highest total DM (Figure 6.2). Total DM produced in autumn was positively influenced by the autumn fertilization (i.e., treatment 3) with an increase of 0.3 t ha^{-1} compared to the N-control treatment (Table 6.3B ; Figure 6.2).

On the contrary, N fertilization had no influence on tiller density (Table 6.3B). An exception was observed during the first year where lower tiller density at the BBCH89 stage was observed for the N-control treatment ($p\text{-value}<0.05$; Figure 6.3).

N fertilization had a significant impact on the height of plants (Table 6.3B). Globally the tallest plants were obtained with the sufficient N treatments (i.e., treatment 3 and 4 ; Table 6.4).

3.1.3. Impact of defoliation

Defoliation was performed in the autumn of 2018 and 2020, its influence was therefore only compared in the growing season of 2019 and 2021. Autumn defoliation globally reduced total DM the following year (Figure 6.4), even if the effect was only significant at the beginning of stem elongation and flowering (i.e., BBCH30 and BBCH65 stages) (Table 6.3A). Autumn defoliation also reduced tiller density at grain maturity (i.e., BBCH89) of 70 tillers m^{-2} (Table 6.3A).

3.1.4. Relationship between aboveground development traits

During the growing season, total DM produced at a given stage had a positive influence on the total DM and tiller density measured at next stages ($p\text{-value}<0.001$). Overall, the spike DM was always positively correlated with total DM and tiller density during the growing season as well as with plant height ($p\text{-value}<0.05$), with the strongest correlations found between spike DM and total DM at the same stage ($p\text{-value}<0.001$). However, total DM at autumn seemed negatively influenced by previous aboveground plant development through a negative correlation with the total DM produced at flowering (i.e., BBCH65 ; $p\text{-value}<0.001$) and with the tiller density during the reproductive phase ($p\text{-value}<0.01$). Correlation coefficients are presented in Table 6.5.

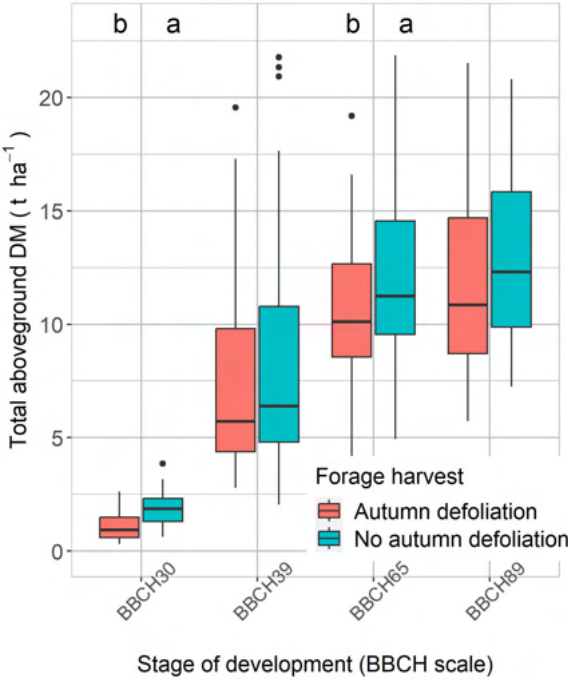

FIGURE 6.4 – Effect of autumn defoliation on the total aboveground biomass during the reproductive phase of 2019 and 2021. Letters represent the results of the post hoc analysis (p-value<0.05) of the effect of autumn defoliation on each phenological stage.

TABLE 6.5 – Correlation coefficients and their significance between vegetative components.

		Total DM						Spike DM		Tiller density		
		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-value*$<$0.05 ; '**' indicating statistical significance at *p-value*$<$0.01 ; '***' indicating statistical significance at *p-value*$<$0.001.

3.2. Grain production

3.2.1. Impact of stand age

Grain yield, spike density, grain density, spike fertility, TKW and grain N content through the different stand ages are presented in Figure 6.5 and marginal means of spike DM, tiller fertility and spike length are provided in Table 6.4. According to the N treatment, stand age had a different impact on grain yield, spike density and grain density (Table 6.3B). For N-low treatments (i.e., treatment 1 and 2), its effect was significant ($p\text{-value}<0.001$) with the highest values observed in 2019 and 2021. The lowest grain yield, spike density and grain density were observed in 2018 and 2020 ($p\text{-value}<0.001$) with means of 0.6 ± 0.1 t ha^{-1}, 204 ± 71 spikes m^{-2} and 6482 ± 889 grains m^{-2}, respectively for the N-control treatment. In contrast, N-sufficient treatments (i.e., treatment 3 and/or 4) permitted to maintain constant yields and some components during the four cropping years with means of 0.9 ± 0.1 t ha^{-1} for grain yield, 485 ± 65 spikes m^{-2} for spike density and 11527 ± 1067 grains m^{-2} for grain density ($p\text{-value}>0.05$; Figure 6.5). Regarding the other parameters, the effect of the stand age was independent of the N fertilization and had a significant impact on tiller fertility, spike fertility, TKW, spike length, spike DM and N content in grains (Table 6.3B). In the first year (i.e., 2018), TKW and the N content in grains were the highest with a mean of 9 ± 0.2 g for TKW as spike fertility was the lowest (Figure 6.5). In 2019, we observed the highest spike DM and spike length and the lowest TKW with a mean of 7 ± 0.2 g (Table 6.4 and fig. 6.5). In 2020, tiller fertility was the lowest with a mean of 56 ± 4 % compared to 74 ± 4 % the other years as spike fertility was the highest with a mean of 31 ± 2 grains $spike^{-1}$ (Figure 6.5 and table 6.4).

3.2.2. Impact of N fertilization

The effect of N fertilization was dependent on the stand age for grain yield, spike density and grain density (Table 6.3B). Concerning grain yield and grain density this effect was significant in 2018, 2019 and 2020 with contrasting results. The effect of N fertilization was positive in 2018 and 2020 and negative in 2019. Contrary to 2018 and 2020, a higher grain yield and grain density was observed in 2019 with the N-control treatment ($p\text{-value}<0.05$; Figure 6.5). Spike density was positively influenced by N fertilization in 2018 and 2021 ($p\text{-value}<0.01$; Figure 6.5). Globally tiller fertility, spike length, spike biomass and N content in grains had reduced level with the N-control treatment contrary to the N-sufficient treatments (i.e., treatment 3 and 4) that increased their levels (Table 6.3B ; Table 6.4 ; Figure 6.5). Spike fertility and TKW were not influenced by N fertilization (Table 6.3B).

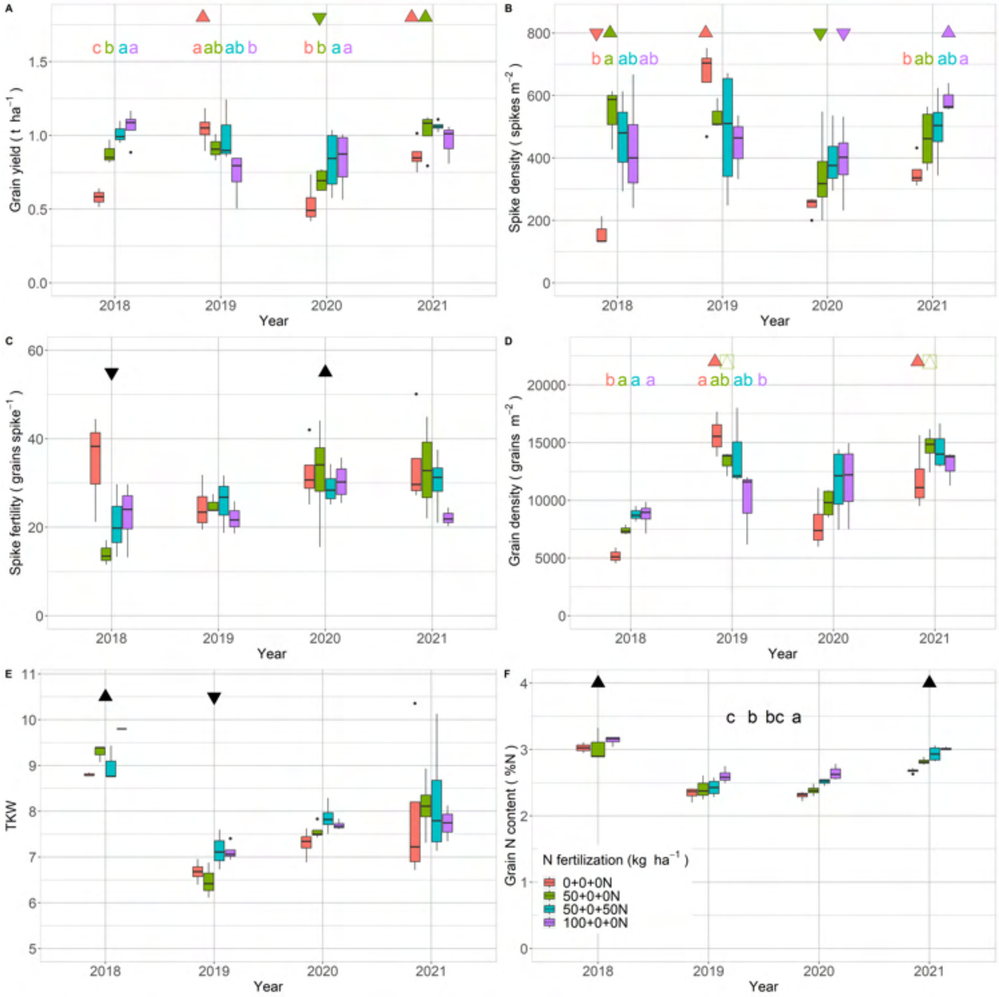

FIGURE 6.5 – Effect of N fertilization and stand age on grain yield, its components and grain N content on the autumn non-defoliated treatment from 2018 to 2021. Letters represents the results of the post hoc analysis (p-value<0.05) of the effect of N fertilization and symbols represents the results of the post hoc analysis (p-value<0.05) of the effect of the stand age. △ specifying an increased value ; specifying an equal increased value ; ▽ specifying a decreased value compared to the other cropping years. The color of the symbols and letters represents the N treatment concerned when there is an interaction between stand age and N fertilization, otherwise the symbols and letters are in black.

3.2.3. Impact of defoliation

Defoliation was only compared in 2019 and 2021, the N content in grains was not measured between defoliation treatments. TKW was the only grain yield components influenced by defoliation in autumn with an increase of 1 g when an autumn defoliation was performed (Table 6.3A).

3.2.4. Partitioning to reproductive organs

Marginal means of spike harvest index, fruiting efficiency, harvest index and N harvest index are presented in Table 6.4. These 4 indicators were influenced by the stand age (Table 6.3B). Spike harvest index, harvest index and N harvest index were reduced in the second year (i.e., 2019) with a mean of 45 ± 9 %, 6 ± 1 % and 31 ± 5 %, respectively. The other years, they averaged 72 ± 9 % for spike harvest index, 11 ± 1 % for harvest index and 55 ± 5 % for N harvest index. Concerning fruiting efficiency, the highest values were observed in 2020 and 2021 with a mean of 143 ± 10 grains g^{-1} (Table 6.4). N fertilization had an impact on the spike harvest index, harvest index and fruiting efficiency. The N-control treatment maximized these three parameters as treatment 4 minimized the spike harvest index and harvest index (Tables 6.3 and 6.4). Defoliation had no impact on these parameters (Table 6.3A).

3.2.5. Lodging

Lodging was observed in 2019 and 2021, just before the flowering stage (i.e., BBCH65 stage). N fertilization increased lodging severity (Table 6.3B) where the N-sufficient treatments (i.e., treatment 3 and 4) presented the highest lodging severity and the N-control treatment the lowest. Defoliation and stand age had no significant impact on lodging (Table 6.3).

Lodging severity was negatively correlated with grain yield (p-value<0.05), harvest index (p-value<0.01), N harvest index (p-value<0.05) and fruiting efficiency (p-value<0.001). This could be explained by the negative impact of lodging on the spike fertility (p-value<0.05), the only grain yield component impacted by the lodging in our study. Lodging severity was positively correlated with many indicators of plant development, including tiller density at the beginning of stem elongation (i.e., BBCH30 ; p-value<0.001), plant height (p-value<0.05) and total DM at previous autumn (p-value<0.05), at beginning of stem elongation, at flag leaf and flowering (p-value<0.001) and at grain maturity (p-value<0.01). Lodging severity didn't have a negative effect on total DM produced the next autumn as the correlation was even positive (p-value<0.05). Correlation coefficients are presented in Table 6.6.

TABLE 6.6 – 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-value*$<$0.05 ; ’**’ indicating statistical significance at *p-value*$<$0.01 ; ’***’ indicating statistical significance at *p-value*$<$0.001.

3.3. Grain yield elaboration

Acceptable fit indices (see section 'Model fitting' of the material and methods) were observed for path analysis that confirms that the specified model fitted well the data. Fit indices and the variance explained are shown in Table 6.7. The path coefficients and their significance are shown in Figure 6.6. Path coefficients are most useful for the comparison of the relative strength of paths within a given path diagram.

The direct (σ_{direct}) and indirect ($\sigma_{indirect}$) effect of crop measurements on grain yield are synthesized in Table 6.8. Grain density was the most positively contributing factor to grain yield with path coefficient $\sigma_{direct} = 0.8$ while σ_{direct} of TKW on grain yield was only of 0.1. Spike fertility and spike density had an indirect positive effect on grain yield via grain density ($\sigma_{indirect} = 0.7$). Tiller fertility and tiller density at grain maturity (i.e., BBCH89) had also a positive indirect effect on grain yield via the increase of spike density and therefore grain density ($\sigma_{indirect} = 0.5$). Spike DM at flowering (i.e., BBCH65) had a small indirect effect on grain yield by an increase of grain density ($\sigma_{indirect} =$ 0.1). Spike length had a weak negative indirect effect on grain yield via a reduction of spike fertility ($\sigma_{indirect} =$ -0.1). This spike fertility is also negatively impacted by spike density resulting in a negative indirect effect of spike density on grain yield ($\sigma_{indirect} =$ -0.5). Spike DM at BBCH89 had a weak positive impact on grain yield by a positive contribution to TKW ($\sigma_{indirect} = 0.05$). Concerning vegetative measurement at beginning of stem elongation (i.e., BBCH30), negative effect was observed on grain yield. Tiller density at BBCH30 had a σ_{direct} of -0.2 and a $\sigma_{indirect}$ via TKW of -0.06 on grain yield, as total DM at BBCH30 had a $\sigma_{indirect}$ of -0.2 via spike fertility and grain density on grain yield. A small negative impact of total DM at BBCH89 was observed on grain yield via a negative influence on TKW ($\sigma_{indirect} = -0.06$). Finally, plant height had a small positive influence on grain yield with σ_{direct} =0.1 and $\sigma_{indirect} = 0.04$ via its positive influence on TKW.

TABLE 6.7 – (A) Fit indices and (B) variances explained of the path analysis.

		(A)	**Model fit indices**				(B)	**(B)**	**Variances explained** (r^2)			
Number of observations	**Degrees of freedom**	**SRMR**	**RM-SEA**	**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

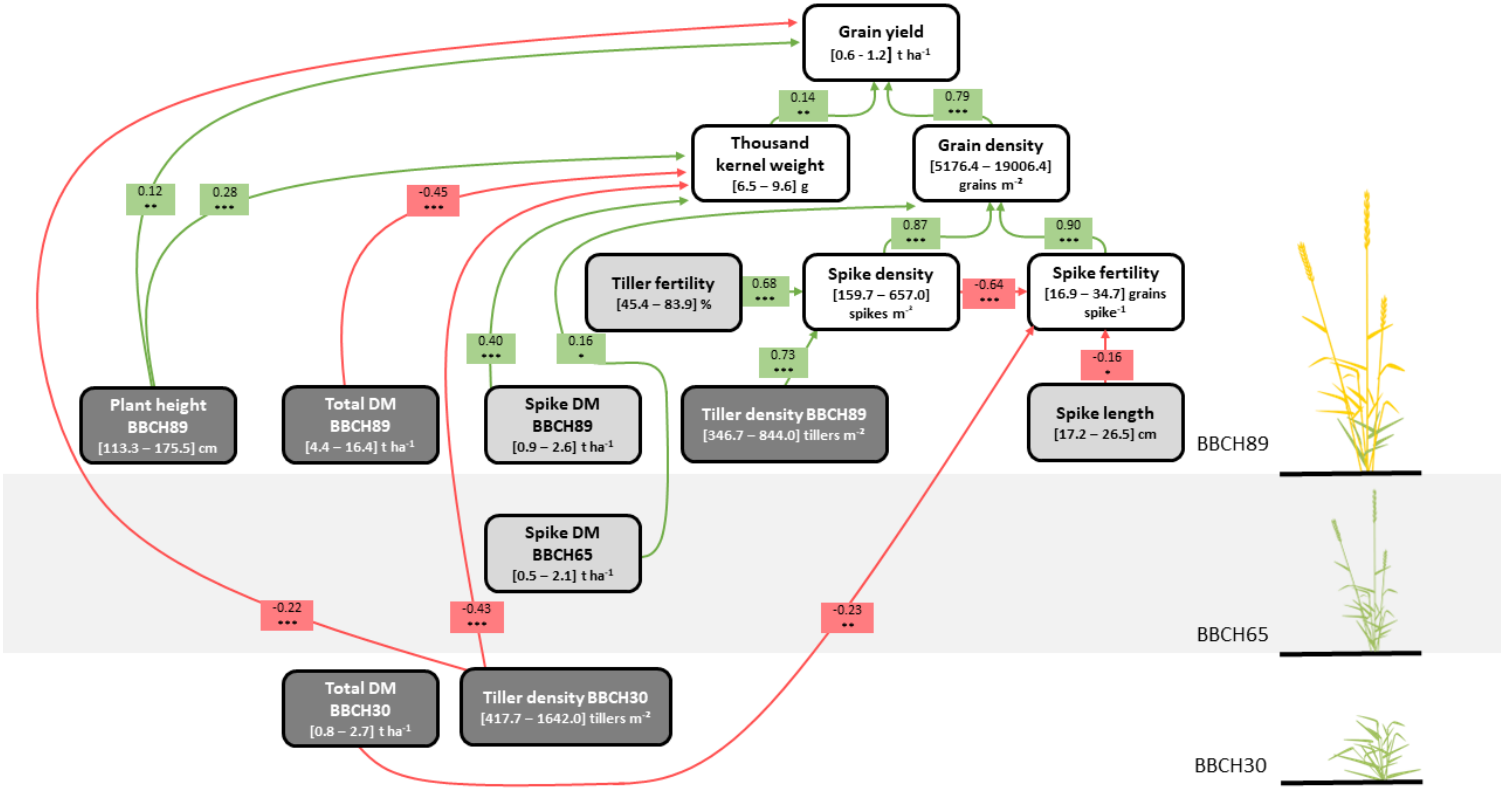

FIGURE 6.6 – Path coefficients of the final model for the relationship between grain yield and crop measurements, followed by their range values between brackets and units. Groups of traits are colored in different shades of gray, including grain yield and its components (in white), maturity traits (in medium gray) and vegetative traits (in dark gray). A direct path is represented by a single arrow that directly connects two traits (e.g., grain yield and grain density), whereas an indirect path is when the path between a pair of traits is separated by another trait (e.g., grain yield and spike density). Insignificant paths are excluded, statistical significance of the path coefficient at p-value$<$0.05 is indicated by "*"; at p-value$<$0.01 by "**" and at p-value$<$0.001 by "***". Green arrows represent positive contributions and red arrows represent negative contributions.

TABLE 6.8 – Significant direct and indirect path coefficients and their significance for crop measurements as they contribute to grain yield.

TKW vs grain yield		
Direct effect	0.14	**
Grain density vs grain yield		
Direct effect	0.79	***
Spike fertility vs grain yield		
Indirect effect via *grain density*	0.71	***
Tiller density at BBCH30 vs grain yield		
Direct effect	-0.22	***
Indirect effect via TKW	-0.06	**
Total DM at BBCH30 vs grain yield		
Indirect effect via *spike fertility* and *grain density*	-0.17	**
Tiller density at BBCH89 vs grain yield		
Indirect effect via *spike density* and *grain density*	0.50	***
Total DM at BBCH89 vs grain yield		
Indirect effect via TKW	-0.06	*
Plant height vs grain yield		
Direct effect	0.12	**
Indirect effect via TKW	0.04	*
Spike DM at BBCH65 vs grain yield		
Indirect effect via *grain density*	0.13	*
Spike DM at BBCH89 vs grain yield		
Indirect effect via TKW	0.05	**
Tiller fertility vs grain yield		
Indirect effect via *spike density* and *grain density*	0.47	***
Spike density vs grain yield		
Indirect effect via *grain density*	0.69	***
Indirect effect via *spike fertility* and *grain density*	-0.46	***
Spike length vs grain yield		
Indirect effect via *spike fertility*	-0.11	0.05

Note. '*' indicating statistical significance at $p\text{-}value<0.05$; '**' indicating statistical significance at $p\text{-}value<0.01$; '***' indicating statistical significance at $p\text{-}value<0.001$.

4. Discussion

4.1. Plant reproductive allocation and allometry

Resource allocation of *Th. intermedium* towards grains was low; grain yield ranged from 0.6 to 1.2 t ha^{-1} (Figure 6.6). Although fruiting efficiency ranged from 76 to 150 grains g^{-1}, values of spike DM at flowering and spike harvest index (i.e., ranging from 0.8 to 1.7 t of DM ha^{-1} and from 45 to 75 %, respectively; Table 6.4), were globally lower compared to mean values of wheat (Hucl and Graf, 1992; Slafer et al., 2015; Terrile et al., 2017; Rivera-Amado et al., 2019). These weak performances primarily resulted from a low yield per spike (considered as a function of TKW and spike fertility). *Th. intermedium* had a limited capacity to set up large grains; the highest TKW was observed during the establishment year with values not exceeding 9.6 g (Figure 6.5). Furthermore, the calculated spike fertility was quite weak ranging from 17 to 35 grains spike^{-1} and remained a major contributor to the yield compared to TKW (Figure 6.6). As observed by Larson et al. (2019) floret fertility of *Th. intermedium* didn't exceed 49 %; this was identified as one of the principal limitations for seed production. Altendorf et al. (2021) also reported the floret site utilization as the primary contributor to the yield per spike. Floret site utilization is a complex trait that can be influenced by different factors such as pollen availability, self-incompatibility, seed shattering or seed abortion (Altendorf et al., 2021). As highlighted by Cattani and Asselin (2018), as a self-incompatible species, selection for uniformity of flowering at optimal time during the day to avoid pollen unavailability and at optimal time during the growing season to avoid post-anthesis heat and drought stress should enhanced the potential for seed set in *Th. intermedium*. Altendorf et al. (2021) also suggested possible resource allocation issues. The N harvest index, as an indicator of the N allocated to the grains compared to the total aboveground biomass, ranged from 31 to 58 % (Table 6.4). In another value range, Mårtensson et al. (2022) found out that the N harvest index of *Th. intermedium* ranged from 17 % to 24 %. Varying among crop species and genotypes of the same species, the N harvest index in wheat is estimated to be more than 80 % and relatively insensitive to the N fertilizer supply (Hawkesford, 2014). In comparison, the utilization of the N by *Th. intermedium* for grain production is not completely efficient. In our previous study (Fagnant et al., 2023), we showed a lack of accumulation of N and DM after anthesis in spikes that can be translated in low grain yields. Previous research on the partitioning of carbohydrate to seed in *Lolium perenne* L. and consequent seed filling suggest that spike has sufficient capacity to fill available seeds without contribution from vegetative tissues (Trethewey and Rolston, 2009). Therefore, more understanding about factors driving the priority of allocation of assimilates and nutrients to each sink during reproductive development of *Th. intermedium* are needed, along with the contribution from each source organs, to enhance its seed sink strength.

From the path analysis (Figure 6.6), compensation mechanisms were observed between yield per spike and yield per plant as spike fertility was negatively influenced by spike density. Pinto et al. (2021) also observed a trade-off with spike weight negatively correlated to spike density of *Th. intermedium*. During the second year, we observed the highest level of tiller density and aboveground biomass at the beginning of the growing season (i.e., BBCH30 stage) compared to the other cropping years (i.e., 1415 tillers m^{-2} and 2.1 t of DM ha^{-1}; Figures 6.2 and 6.3). However, such an early biomass and tillering productivity was found detrimental to the yield per spike through spike fertility and TKW (Figure 6.6) and can further increase the lodging risk (Table 6.6). The path analysis also revealed that spike length, which was also the highest during the second growing season, had a negative influence on spike fertility. This year, the lowest values of TKW and spike harvest index were observed, and N harvest index and harvest index dropped to values of 31 % and 6 % (compared to a harvest index of 11 % the other years), respectively (Table 6.4). This indicated a general relative limited allocation of resources to grain production, and a loss of allocation to seeds on a dry weight basis when the biomass is peaking. As explained by Slafer et al. (2022), initial overproductions of organs in wheat - such as tillers - or floret primordia - were followed by their massive mortality. We hypothesize that this negative relationship arises from feedback interactions induced by the competition for a shared limiting growth resource. So, allocating more resources to the survival and growth of many reproductive tillers, hence increasing spike density, implies fewer resources for each individual tiller and spike, and limits their fertility (Yang et al., 2019). In our case, during the second growing season, tillering of *Th. intermedium* was undoubtedly excessive. Many tillers couldn't be supported up to grain harvest and regressed or were weakly productive. However, contrary to Hunter et al. (2020a) spike fertility in this study didn't decrease over time that could be explained by our limited spike density (i.e., 660 spikes m^{-2} maximum) compared to Hunter et al. (2020a) that observed up to 960 spikes m^{-2}. This spike density was mostly linked to the density of tillers subsisting until grain harvest (Figure 6.6) and was relatively constant over time (Figure 6.5). The lower spike density of this study may have limited competition between spikes and maintained relatively higher spike fertility. All in all, this sustains the hypothesis that yield and harvest index decline with stand age such as observed in other studies (Jungers et al., 2017; Pugliese, 2017; Frahm et al., 2018; Tautges et al., 2018; Bajgain et al., 2020; Locatelli et al., 2023) might partly be caused by the increasing competition in stands with excessive tiller density (Law et al., 2020; Cassman and Connor, 2022). While tillering is necessary for aboveground biomass production, selection for grain yield improvement should focus on the reduction of excessive tillering (incl. unfertile tillers) as also highlighted by Altendorf et al. (2021) to allow greater resources allocation to the yield per spike through spike fertility and TKW.

4.2. Key drivers of the final grain yield in field

Overall, our study demonstrates for the first time a potential to achieve constant grain yields over four years, with yields at the high end of the range of values known for this crop (Figure 6.5). The grain density was the major contributor of the final grain yield per hectare (Figure 6.6), as found with cereal crops (Hay and Porter, 2006). Grain density of *Th. intermedium* was almost evenly associated to spike density and spike fertility (Figure 6.6). It was positively related to the biomass of spikes reported at the flowering stage (i.e., BBCH65 stage ; Figure 6.6). Actually, the biomass of the spike at anthesis is known to be a strong proxy of grain density since it indicates a long stem elongation phase, which is often ultimately associated with an increase in seed number. In the absence of stress or other factors affecting grain set, cereal grain yield is source limited with a defined capacity to store assimilate within each grain. The available biomass from post-flowering photosynthesis or from reserves is then allocated to grains up to this limit (Hay and Porter, 2006; Slafer et al., 2022).

To maintain grain density and final yield over the years, spike density (reproductive tillers) remained a strong driver since spike fertility ranged similar values from one year to another and TKW had little variability (Figure 6.5 and Figure 6.6). However, we observed that when the tiller density is too high and/or resources too scarce, significant mortality and inhibition of reproductive growth of tillers can occur (Figures 6.3 and 6.5). This was demonstrated in grasses (Meijer and Vreeke, 1988; Svečnjak et al., 2022) and has already been observed with *Th. intermedium* (Hunter et al., 2020a). Lower grain yields were found linked to a lower number of spikes and the subsequent reduction in the number of grains under the resource-limited conditions. Resource limitations came either from N (non-fertilized plots ; Figure 6.5) or water deficiencies (see Table 6.2 third year with the lower water reserve during the entire growing season ; Figure 6.5). The importance of reproductive tillers had also been highlighted on the yield per plant in the study of Altendorf et al. (2021), where the number of spikes, measured on a per-plant basis, was identified as the primary contributor of yield. Therefore, we hypothesized that in the high-yielding environment of our experiment, i.e., deep and fertile soil conditions coupled with sufficient N fertilization (i.e., 100 kg N ha^{-1} over the growing season) and combined with a good stand establishment (i.e., from 500 to 1000 tillers m^{-2} at the beginning of the growing season; Figure 6.3) and no overproduction of spikes (i.e., 660 spikes m^{-2} maximum) the crop is able to set up a sufficient number of grains to maintain grain yield potential as stand ages.

4.3. Agronomic levers to enhance grain yield

4.3.1. N fertilization

Variable yield responses to N fertilization have previously been reported in the literature, either with increases (Zimbric et al., 2021), decreases (Frahm et al., 2018) or no impact on *Th. intermedium* grain yield (Dobbratz et al., 2023). In our study, the positive grain yield response to N fertilization was found to be mainly related to spike density, tiller fertility and spike DM. Indeed, N fertilization levels around 100 kg N ha^{-1} (either fully applied in early-spring (i.e., BBCH29) or split between autumn and BBCH29) led to spike densities roughly above 400 spikes m^{-2}. However, in the second year the fertilization of 100 kg N ha^{-1} fully applied in early-spring stage was detrimental to grain yield. Meijer and Vreeke (1988) showed that grain yield responses to N fertilization of three perennial grasses (i.e., *Lolium perenne*, *Poa pratensis* and *Festuca rubra*) was mainly related to the number of inflorescences produced and to a lesser degree to the production of seed per inflorescence. In fact, the timing of N availability must be thought accordingly to the tillering dynamic of the species or genotype grown. Langer (1980) highlighted that seed yields of grasses depend strongly on spike density, and early formed tillers are largely responsible for producing these spikes. For example, with *Dactylis glomerata* L., *Phleum pratense* L. and *Festuca pratensis* Huds. the tillers present in early autumn make up the bulk of the grain yield the following year. However, with *L. perenne* it seems to be possible for a reasonably large proportion of spring-formed tillers to become fertile (Langer, 1980). In addition, early formed inflorescences of *L. perenne* consistently had more spikelets per ear and flowers per spikelet when compared to late-formed ones (Svečnjak et al., 2022). In the BE2 experimental site (Table 4.2), multiple sowing dates were tested (i.e., last spring : 15^{th} May 2019, late summer : 23^{rd} August 2019 or autumn : 3^{rd} October). We observed the same number of tiller in the next summer between the different sowing dates. However, a lower aboveground biomass production coupled with a lower tiller fertility and grain yield was observed with the October sowing as opposed to the May sowing as shown in supplementary material (Table 6.9). This underline the importance of early induced tillers for *Th. intermedium* to maximize grain yield, as the reproductive potential of the tillers produced from the earliest sowing (i.e., May) was the highest. So, it is critical to deepen the research on the dynamic of both tillering and N availability to help finding the right conditions for the enhancement of reproductive tiller growth and the mitigation of trade-off with the yield per spike. In this way, study the unique application of N in autumn in comparison to a unique application in spring would be helpful. In our study, N fertilization scheme including 50 kg ha^{-1} applied in autumn combined with 50 kg ha^{-1} applied in early-spring (i.e., BBCH29) (i.e., treatment 3) appears to be a good compromise for optimal grain production by strengthening early tillering in autumn, and sustaining tiller fertility later on, while limiting the risk of excessive biomass production. Still, this rule of thumb should be adapted depending on local conditions and the observed crop development in field. For instance, when *Th. intermedium* shows an important biomass production at the beginning of the season (BBCH30), and the number of tillers is already enough (i.e., tiller density around 1300 tillers m^{-2}), we believe that the early-spring N fraction should be reduced to avoid overproduction of tillers and later mortality or competition effects.

4.3.2. Management of post-harvest crop residues

Few days after each harvest of grains, the straw was exported from the field, and the post-harvest crop residues were shredded at 5 cm from the ground to prevent obstruction of light perception at the bases of *Th. intermedium* plants. This management operation might be at play to explain the relatively constant fertility of the tillers (Table 6.4). Indeed, previous studies have highlighted a decline in yield with stand age, combined with a decrease in spike density (Jungers et al., 2017; Law et al., 2020). Authors hypothesized that changes in light quality perceived by the axillary buds located at the bases of the plant, which could reduce reproductive tiller initiation or trigger light avoidance syndrome, might be the cause (Jungers et al., 2017; Pinto et al., 2021; Rouet et al., 2021). Different management practices to increase light penetration to the canopy have previously been tested to maintain *Th. intermedium* grain yields over the long term, such as row spacing (Hunter et al., 2020a), between-row plant termination methods (with cultivation or herbicide application ; Bergquist (2019); Law et al. (2020); Pinto et al. (2021)) or within-row suppression methods using burning and mowing (Bergquist (2019); Hunter et al. (2020a); Pinto et al. (2021)). Inconclusive results were observed : generally, such agronomic practices had the potential to increase yields, but not to prevent yield decline with stand age. In addition, these operations at plot scale could increase tiller and spike fertility, but they were associated with a reduction of other yield components such as spike density, inducing an effective increase of the yield per row without increasing the yield per surface unit (Pinto et al., 2021). As observed for other species, the possibility that *Th. intermedium* tillers - present since early autumn - would become the future reproductive tillers having the major impact on grain yield the following year cannot be excluded (Langer, 1980). The optimum time of light perception at tiller bud level for the induction of reproductive tillers and the resulting management practices in link with tiller population regulation remain to be identified with the construction of new knowledge about the tiller fertility dynamic of *Th. intermedium*.

4.3.3. Autumn defoliation

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

5. Conclusions

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

6. Supplementary material

TABLE 6.9 – Plant density, total aboveground biomass, plant height, tiller and spike density, tiller fertility and grain yield of the different sowing dates of *Th. intermedium* after stand establishment of 2019 and during the two first grain production years (i.e., 2020 and 2021).

	2019			**2020**			**2021**		
	15th May 2019	**23rd August 2019**	**3rd October 2019**	**15th May 2019**	**23rd August 2019**	**3rd October 2019**	**15th May 2019**	**23rd August 2019**	**3rd October 2019**
Plant density (plants m^{-2})	358 ± 20	366 ± 20	389± 20						
Total aboveground DM at BBCH89 (t of DM ha^{-1})				14.2 ± 0.6 b	12.7 ± 0.6 b	5.5± 0.6 a	14.7 ± 0.6 b	14.3 ± 0.6 b	11.6 ± 0.6 a
Plant height at BBCH89 (cm)				169 ± 3 c	154 ± 3 b	106 ± 3 a	165 ± 3	163± 3	164± 3
Tiller density at BBCH89 (tillers m^{-2})				608 ± 52	606 ± 52	516 ± 52	839 ± 56 b	768 ± 52 ab	610 ± 52 a
Spike density at BBCH89 (spikes m^{-2})				589 ± 47 b	555 ± 47 b	240 ± 47 a	657 ± 51	692 ± 47	580 ± 47
Tiller fertility at BBCH89 (%)				97 ± 2 b	92 ± 2 b	47 ± 2 a	79 ± 3 a	90 ± 2 b	95 ± 2 b
Grain yield (t ha^{-1})				1.9 ± 0.1 c	1.1 ± 2 b	0.4 ± 2 a	/	/	/

7

Potentiel de production fourragère de *Th. intermedium*

1. Production fourragère sous une gestion double usage

Introduction au chapitre

Cette première section du chapitre 7 est adaptée d'une publication dans le journal 'Grass and Forage Science' :

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Le double usage de la culture nécessite d'étudier le potentiel de rendement en grains en fin de saison de croissance mais également le potentiel fourrager de *Th. intermedium*. Cette section vise donc à étudier ce potentiel fourrager, tant en termes de quantité qu'en termes de qualité, de la biomasse produite par *Th. intermedium* sur une saison de croissance dédiée à la production de grain. Diverses associations aux légumineuses ont également été testées. Cette section apporte des éléments concernant les possibles valorisations fourragères et la capacité de l'espèce à gérer la compétition des légumineuses.

Forage potential of *Thinopyrum intermedium* through near-infrared spectrometry and grown in mixture with various legumes.

Fagnant L.[1*], Duchene O.[2], Bindelle J.[3], Beckers Y.[3],Decruyenaere V.[4], Dumont B.[1]

*Corresponding author : laura.fagnant@uliege.be ; +3281622141

[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

[3]ULiege - Gembloux AgroBio-Tech, Precision livestock and nutrition laboratory, B- 5030 Gembloux, Belgium yves.beckers@uliege.be ; Jerome.Bindelle@uliege.be

[4]CRA-W, Animal production Unit, 8 rue de Liroux, B- 5030 Gembloux, Belgium v.decruyenaere@cra.wallonie.be

Credit authorship contribution statement
L.F. : conceptualization, planning of the experiments, formulation of research goals and aims, development and design of methodology, carrying out the samplings, data curation, formal analyses (statistical and mathematical), interpretation of result, data presentation and visualization, writing – original draft preparation.
B.D. : supervision, development and design of methodology.
Y.B., V.D., : help provided for data presentation and vizualization, contributed to the interpretation of result.
Y.B., V.D., O.D., J.B., B.D. : critical review, commentary and revision, validation.
Note : chemical analyses were performed by Sylvie Mabille, Chloé Dufrane, Antonin Guillaume and Lucas Villé.

Keywords
Perennial grain, *Thinopyrum intermedium*, Intermediate wheatgrass, Kernza®,

Forage evaluation, Legume intercropping.

Abstract

Intermediate wheatgrass (IWG) [*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey] is a perennial grass, dual-purpose crop that can provide environmental services. In addition to the grain production, assessing its forage potential is crucial. We developed models for near-infrared spectrometry (NIRS) prediction of the chemical composition and digestibility of IWG across various experimental sites. Among these, a Belgian field was used to compare its dual production in pure stands or in mixture with legumes. Good quality near-infrared (NIR) predictions were observed, offering an efficient tool to characterize the forage composition of IWG. Its forage parameters were mainly influenced by the phenological stage with an increase of fibers and a decrease of protein, digestibility, and energy content (net energy for lactation (NE_L)) during the growing season. IWG forage at vegetative stages could be used to feed lactating dairy cattle with a NE_L of 1625 kcal kg^{-1} of DM but, its biomass was low averaging 1.8 t of DM ha^{-1}. At grain maturity, biomass was higher (i.e., 5.3 t of DM ha^{-1}), representing 73 to 92 $\%$ of the total biomass production, and could replace straw in high-starch dairy diets with a NE_L averaging 849 kcal kg^{-1} of DM. Although the mixture of IWG with legumes enhanced some forage parameters, its value as animal feed was not improved. In mixture, we observed a tradeoff between the increase of the forage yield and the reproductive potential of IWG. These insights can inform the on-going process of breeding and help farmers to design relevant systems to experiment this new crop.

1.1. Introduction

To reconcile the objective of dedicated land to arable cropping for human food consumption and grasslands area for ecological intensification, IWG [*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey] is currently proposed as a dual-use perennial grass. The crop can provide both forage and grain as well as other ecosystem services thanks to its year-round soil cover and its persistent root system (Crews et al., 2016; Rasche et al., 2017; Ryan et al., 2018). Due to its actual low grain yield (Fagnant et al., 2024), forage valorization could be a way to generate additional agricultural production to increase crop profitability (Bell et al., 2008; Larkin et al., 2014; Newell and Hayes, 2017). Forage harvesting can be achieved at different time during the year depending on the farmer's objectives. While grains and straws are harvested in summer, additional cuts or grazing operations could be performed in autumn, early-spring, or both for forage production (Culman et al., 2023; Hunter et al., 2020b). However, knowledge of the forage quality is essential to foster an adequate use of unconventional forage resources such as *Th. intermedium* in the feeding system of domestic herbivores.

Pasture species with high feeding value can increase farm profitability and reduce externalities through the improvement of feed autonomy (Thomas et al., 2021). Chemical composition, intake, digestibility, and efficiency of utilization are the main features determining the nutritive value of a given forage. Notably, the fiber, lignin, and protein contents of forage tissues are associated with voluntary feed intake and digestibility properties (Cherney, 2000). Deriving forage quality from multiple chemical analyses is time-consuming. The NIRS is successfully used since multiple decades as a non-destructive alternative to lab reference methods. Nevertheless, a crucial calibration step is required to relate the NIR optical measurements to the desired constituent or property used to define the nutritional quality of forages (Deaville and Flinn, 2000). As for other grasses, *Th. intermedium* forage quality depends on its phenological stage at harvest. Early in spring, before or at the beginning of the reproductive growth, the proportion of leaves is significantly higher than that of stems and represents more than 80 % of the aboveground biomass (Barriball, 2020; Fagnant et al., 2023). After this stage, the proportion of stems increases until spikes are fully emerged. At grain maturity, leaves only represent 13 % of the aboveground biomass, while stems and spikes are respectively 71 % and 16 % of it (Fagnant et al., 2023). This progressive change during the growing season is linked with an increase in total aboveground biomass but is known to lead to a significant decrease in forage quality (Thomas et al., 2021; Wróbel et al., 2023).

Agronomic management of *Th. intermedium* can help to increase the global forage yield potential in terms of quantity and quality. In this way, additional forage harvesting in spring, autumn or both increases the total production of biomass of *Th. intermedium* compared to a unique harvest at grain maturity (Hunter et al., 2020b). In the study of Culman et al. (2023), harvesting forage in the spring resulted in the lowest forage biomass but with the highest forage quality, autumn harvest was intermediate in terms of biomass and quality and summer harvest maximized biomass but resulted in the lowest quality. Although the forage quality at grain maturity (i.e., straw) is relatively poor, Hunter et al. (2020b) highlighted that the higher quantity of biomass harvested is of great interest as it provides a tangible second outcome besides grains. The impact of forage harvesting, particularly the timing and frequency, on grain yields also needs to be considered. As observed by Culman et al. (2023), harvesting forage in the summer and autumn increased grain yield as harvesting forage in the spring reduced grain yield. Besides forage harvesting, the mixture of legumes with forage grasses is known to secure forage yield potential (Louarn et al., 2016) and can improve forage quality. Compared to pure grass forage, protein content can be reinforced, the fiber content lowered (Baumont et al., 2016) and the digestibility and therefore the energy value enhanced, according to the legume species and its chemical and morphological traits. For example, the digestibility of legumes such as lucerne or red clover (i.e., *Medicago sativa* L. and *Trifolium pratense* L.) is generally lower than or equivalent to that of grasses. By contrast, white clover (i.e., *Trifolium repens* L.) stands out for its very high digestibility, superior to that of grasses, due to its notable absence of stems and lower fiber content. In addition, legume digestibility decreases less rapidly over the growing cycle than that of grasses, so their nutritional value is more stable over time (Baumont et al., 2016). In the study of Favre et al. (2019), the forage provided by the mixture of *Th. intermedium* with red clover tended to have lower fiber and higher protein contents compared to a *Th. intermedium* monoculture and increased forage yield. As a dual-use crop, a range of effects of the legume mixtures on the grain yield of *Th. intermedium* is documented. Some experiments showed lower grain yields in lucerne or red clover mixtures than monoculture (Favre et al., 2019; Mårtensson et al., 2022; Pinto et al., 2022; Tautges et al., 2018). Other experiments demonstrated similar grain yields in lucerne, berseem clover (*Trifolium alexandrinum* L.), kura clover (*Trifolium ambiguum* M. Bieb), sweet clover (*Melilotus officinalis* L.), and white clover mixtures than monoculture (Dick et al., 2018; Pinto et al., 2022; Reilly et al., 2022; Tautges et al., 2018). Thus, the interaction between the species within the mixture needs to be studied to favor complementary and reduce competitive relationships.

In this study, we aimed to develop models for NIRS prediction of the chemical composition and enzymatic in vitro digestibility of *Th. intermedium* forage. Through the speed of analysis, such model should facilitate the characterization of forage nutritive value of this novel multifunctional species where efforts are still ongoing to describe and improve its dual-use productions. Secondly, using the developed prediction models, we evaluated the grain and especially the forage production of the crop, either in monoculture or in mixture with different legume species.

1.2. Materials and methods

1.2.1. Experimental sites

To characterize the chemical composition and enzymatic in vitro digestibility of the forage of *Th. intermedium* (IWG) through near-infrared spectra models, samples from different experimental sites were used to cover a wide range of pedoclimatic conditions, agronomic managements, crop ages and phenological stages (Table 7.1).

Data collected on the BE3 experimental site in Belgium (Table 7.1) was used to characterize the forage production of *Th. intermedium* under a dual-use management (i.e., spring and autumn forage harvest coupled to grain and forage harvest at grain maturity). This field experiment was conducted during two successive cropping years using a complete randomized split-plot design (4 x 8 m subplots) with a forage harvest factor as the main-plot treatment and a species mixture factor as the split-plot treatment, with four replicates. The forage harvest factor compared different treatments, not studied in this study (Table 7.2). An autumn forage harvest was performed both years while spring harvest was only performed in the second year due to insufficient plant establishment in the first year. During these mechanical forage harvests (i.e., spring and autumn forage harvest), the aboveground biomass was cut to a height of 7 cm (i.e., above the apex height) and exported from the field. In this experiment, four treatments compared the effects of the mixture with different legumes species : I) *Th. intermedium* monoculture (IWG), II) *Th. intermedium* in mixture with white clover, III) *Th. intermedium* in mixture with red clover and IV) *Th. intermedium* in mixture with lucerne. Legumes were seeded in the interrow of *Th. intermedium*, but only on the half of all interrow to reduce light competition as described in Figure 7.1. Each subplot (i.e., 4 x 8 m) was split in two : one plot was dedicated to destructive sampling during the growing season and the other one to grain yield measurement in summer.

TABLE 7.1 – Detailed information about the experimental sites, their design and their management.

		Experimental sites					
Site code :		***BE1***	***BE2***	***BE3***	***FR1***	***FR2***	***FR3***
Location :							
Country		Belgium			France		
GPS Long. (DD)		4.7063	4.7052	4.7091	5.1251	5.0920	5.143
GPS Lat. (DD)		50.5664	50.5659	50.5652	45.4250	45.2746	45.3323
Soil type :		Clay loam			Loam	Sandy-loam (stony)	Sandy-loam
Climate :							
Average annual rainfall (mm)		852			881	984	983
Average annual min temperature (°c)		7			7.8	6.3	6.3
Average annual max temperature (°c)		14.2			16.5	16.1	16.1
Type of experiment :		Research station (microplots) Randomized split-plot design (4 replicates)			On-farm experiment Strips design (3 replicates)		
Implementation :							
Sowing date		22-09-2017	15-05-2019 23-08-2019	09-09-2021	20-09-2017	15-09-2018	05-09-2017
Seed population		The Land Institute (TLI-C5)			The Land Institute (TLI-C5)		
Seeding rate (kg/ha)		20		12	18		
Interrow spacing (cm)		25	12,5 or 25	28	25	12	20
Field management :							
N fertilization (kg N/ha)	*BBCH30*	0 or 100	50	50	50	50	50
	BBCH39	0 or 50	50	0	0	0	0
	Autumn	0 or 0	50	50	0	0	0
Weeding		Chemical + mechanical		Mechanical	/	/	/
Crop protection		/		/	/	/	/
Post-harvest residue management		Chipping or mowing at 5cm from the ground					
Cropping year for data collection :		2020*, 2021	2019*, 2020, 2021	2022, 2023	2021	2021	2021
Growing stages for data collection (BBCH scale) :		BBCH2., BBCH30, BBCH39, BBCH65, BBCH89			BBCH29, BBCH30, BBCH65	BBCH29, BBCH30	BBCH29, BBCH30, BBCH65

Note. : * is indicating that only the autumn vegetative stage was collected this year. BBCH2. is corresponding to the autumn vegetative stage ; BBCH29 to the spring vegetative stage ; BBCH30 to the stem elongation stage ; BBCH39 to the flag leaf stage ; BBCH65 to the flowering stage and BBCH89 to the grain maturity stage.

TABLE 7.2 – Factors tested in the BE3 experimental site during the two cropping years.

Management treatments	Cropping year where treatment was studied	
(A) Forage harvest factor	2022	2023
Unique forage production : multiple cuts		x
Dual production : spring cut + grain harvest + autumn cut		x
Dual production : grain harvest + autumn cut	x	x
(B) Species mixture factor		
Th. intermedium monoculture (IWG)	x	x
Th. intermedium in mixture with white clover (IWG+ WC)	x	x
Th. intermedium in mixture with red clover (IWG+RC)	x	x
Th. intermedium in mixture with lucerne (IWG+Lu)	x	x

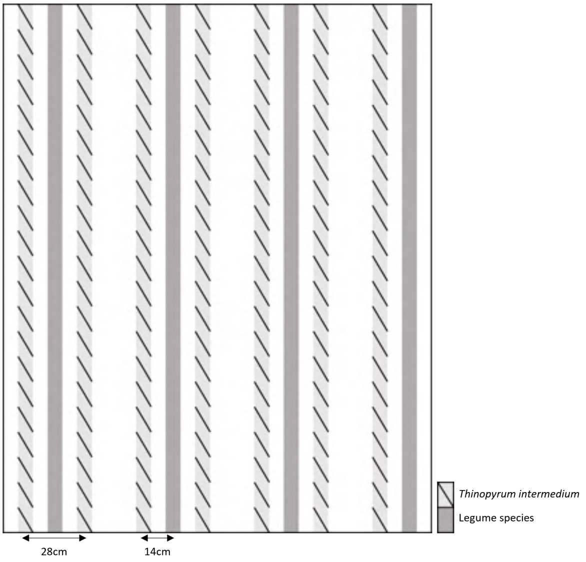

FIGURE 7.1 – Interrow disposition in subplots between *Th. intermedium* and the different legume species in the BE3 experimental site.

1.2.2. Data collection

For all experimental sites, aboveground biomass was sampled during the growing season with a 50 x 50 cm quadrat and cut at 5 cm above soil surface. Fresh samples were weighted to obtain the fresh matter and then oven-dried (72 h at

60 °C) and weighted again to obtain dry matter (DM60). Samples were collected at different phenological stages, rated with the BBCH scale (Meier, 2018), from the vegetative stage (BBCH2.) to the grain maturity stage (BBCH89) as mentioned in Table 7.1 and illustrated in Figure 4.7. Samples were then ground to a particle size of 1 mm in a FOSS Tecator Cyclotec 1093 mill; Foss company; Hillerød; Denmark.

The dried and ground samples were subjected to near-infrared radiation with wavelengths ranging from 400 to 2498 nm by using a XDS Monochromator Type XM-1000 FOSS spectrometer; Foss company; Hillerød; Denmark. The spectral data were recorded with a step of 2 nm. The spectral absorbance values were recorded as log (1 R^{-1}), where R is the sample reflectance.

1.2.3. Development of NIR spectra models

1.2.3.1. Chemical analyses

To develop NIRS prediction models for the chemical composition and enzymatic in vitro digestibility of the forage of *Th. intermedium*, 223 samples covering the variation range within the database (Table 7.1) were selected to undergo the chemical reference analyses.

The parameters described below were expressed on a dry matter basis (method 967.03; AOAC, 1990). The total ash content was determined by weighing the sample fraction remaining after complete calcination at 550 °C (method 942.05; AOAC, 1990). The crude protein (CP) was calculated as total Kjeldahl N multiplied by 6.25 (method 981.10; AOAC, 1990). Fiber contents were analyzed with the Fibercap system (Foss Electric, Bagsvaerd, Denmark). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) (method 973.18; AOAC, 1990) contents were determined as described by Van Soest et al. (1991). The NDF was analyzed using Termamyl (Novo Nordisk, Bagsværd, Denmark). The acid detergent lignin (ADL) was analyzed according to Van Soest (1963) and the crude cellulose (CEL) according to Weende (method 978.10; AOAC, 1990). The enzymatic in vitro digestibility of organic matter (OMD_{cel}) was analyzed according to De Boever et al. (1986). All analyses were performed with two replicates. Some sample masses were insufficient to perform all chemical analyses, inducing a lower number of observations for the OMD_{cel} parameter (i.e., 126 observations compared to more than 200 observations for the other chemical parameters; Table 7.3).

1.2.3.2. Characterization of the database

To identify the factors underlying the variability within the database composed of the different chemical parameters and digestibility of *Th. intermedium* forage (section 1.2.3.1), a principal component analysis (PCA) was conducted. The *PCA* function of R program version 4.1.2 (R Core Team, 2024) was used on centered and scaled data.

1.2.3.3. Predictive models and assessment of their quality

All the following analyses were performed on the MatLab R2018a software. First, the NIR spectra of the 223 samples were subjected to pre-processing to remove noisy regions. The *SNV* (Standard Normal Variate) function of Matlab was firstly performed to reduce the effects of interference related to the dispersion and particle size of the sample (Eylenbosch, 2018). Then, the detrend function was used to reduce the curvature and offset of the spectra. Finally, the Savitzky-Golay algorithm was applied allowing for curve smoothing and background reduction (Eylenbosch, 2018).

For each forage parameter (Ash, CP, NDF, ADF, ADL, CEL, OMD_{cel}), a partial least squares (PLS) regression was performed on the pre-processed spectra (shown in Figure 7.4) with an explanation of the variance set at 50 %. The standard coefficients (i.e., β-coefficients) from the PLS regression were used to identify the most significant wavelengths to explain the variability within the parameter values (Eylenbosch et al., 2018). If significant wavelengths of similar sign were too close (i.e., distance set at 25 nm), only the wavelength with the highest β-coefficient was kept. Finally, these wavelengths were fed into a multiple linear regression to predict the different forage parameters. Of these 223 analyzed samples, 70 % were randomly selected to calibrate the models. The remaining 30 % samples were used for model validation as an internal validation (Table 7.3).

To evaluate the model quality, the following criteria were used : the modelling efficiency (EF) (or the Nash-Sutcliffe model efficiency coefficient), the standard error of calibration (SEC), the standard error of prediction (SEP) (i.e., when the validation is performed on a set of independent samples) and the ratio of the standard deviation (RPD) (i.e., standard deviation (SD) between the database reference values and the SEP). To define a model as acceptable, EF value should reach at least 0.5, the SEC value should be as low as possible and close to the SEP and finally, the RPD should be greater than 3 (Beaudoin et al., 2008; Minet et al., 2018; Murphy et al., 2022).

1.2.3.4. Productivity assessment on the BE3 site

- Forage productivity assessment

To assess the forage production of *Th. intermedium* in a dual-use management and compare the effect of the mixture with different legume species, the data was collected from the BE3 experimental site (Table 7.1). As described in section 1.2.2, samples of aboveground biomass were collected during the growing season to quantify forage biomass (in DM) of each species separately (i.e., *Th. intermedium*, white clover, red clover and lucerne). Spectral data were collected alongside to predict their forage chemical composition and digestibility. Forage parameters of *Th. intermedium* were derived from the predictive model described in section 1.2.3 as forage parameters of legumes were derived from a referenced spectral database as described by Minet et al. (2018). To go further in the characterization of the forage composition, the organic matter (OM) content of the samples was determined as the percentage of dry matter excluding the ash content. Specifically for *Th. intermedium*, the crude fat content was analyzed on 26 samples selected to capture the different phenological stages (i.e., diethyl ether extraction with a Soxhlet device, method 920.39 ; AOAC, 1990) to assign the crude fat content at each phenological stage (i.e., BBCH2. and BBCH30 : 30 g kg^{-1} of DM, BBCH39 : 25 g kg^{-1} of DM and BBCH65 and BBCH89 : 20 g kg^{-1} of DM). The nonfibrous carbohydrates (NFC) content was calculated by removing the crude fat, the CP and the NDF content from the OM content. Finally, from the predicted forage parameters, the NE_L of the different legume species and *Th. intermedium* was calculated according to the Dutch feed evaluation system for ruminants (CVB, 1991). Depending on the forage composition of samples (i.e., ash, fiber content, OMD_{cel} or CP), various feed equations can be used to estimate NE_L values. Following the PCA analysis (see section 1.2.3.2 and 1.3.1.1), the composition of *Th. intermedium* varied with the phenological stage of the crop. Different equations were used to estimate the NE_L value of *Th. intermedium* : ‘fresh grass’ equations for the vegetative stages (i.e., BBCH2. and BBCH30) ; ‘hay’ equations for the BBCH39 stage and ‘straw’ equations for the BBCH65 and BBCH89 stages. The NE_L values of legumes were all calculated with the ‘fresh grass’ equations.

The different forage parameters (i.e., OM, CP, CEL, NDF, ADF, ADL, OMD_{cel}, NE_L) of the grass-legume mixture were then calculated as the weighted average of *Th. intermedium* and the legume species based on their respective DM proportion of the total mixture DM.

- Grain yield productivity assessment

From BBCH30 to BBCH89 stages, tillers and spikes, when present, were counted from the aboveground biomass samples of *Th. intermedium* (as described in section 1.2.2.) to estimate tiller and spike density. At grain maturity, plots were harvested with a trial combine harvester to obtain grain yield on a DM basis and cleaned, but unsorted seeds basis (i.e., a mix of hulled and dehulled seeds).

- Standard statistical analysis

All data analyses were conducted in the R program version 4.1.2 (R Core Team, 2024). As spring forage harvest was only performed in the second year, an ANOVA was performed for each cropping year separately. Within the different ANOVA, mixed models were applied with *lmer* function from the *lme4* package. Two-way ANOVA was performed with the species mixture and the phenological stage considered as fixed effect, while replicates as random effect. Models were evaluated to ensure they met the assumptions of independence and normality of residuals through the *plotresid* function. Transformation of variables was not necessary as the assumptions were met. Following the ANOVA, 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. Grain yield, tiller density and spike density of *Th. intermedium*, aboveground biomass of *Th. intermedium*, legumes and the grass-legume mixture, as well as their forage parameters (i.e., OM, CP, CEL, NDF, ADF, ADL, OMD_{cel}, NE_L) were the analyzed variables.

1.3. Results

1.3.1. Prediction of *Th. intermedium* forage parameters

1.3.1.1. Database characterization

The performed principal component analysis is illustrated in Figure 7.2, OMD_{cel}, CP and fiber parameters (i.e., ADF, NDF and CEL) were relatively well represented by the first principal components (PC1), explaining 67.5 % of the variance. As expected, principal component analysis indicated that there was a clustering effect on the database through phenological stages. Globally, vegetative stages (i.e., BBCH2., BBCH29 and BBCH30) were on the negative axis of the PC1 indicating higher OMD_{cel}, CP and lower fiber content, while late reproductive stages (i.e., BBCH65 and BBCH89) had the opposite behavior. The flag leaf stage (i.e., BBCH39) represented an intermediate situation

(Figure 7.2).

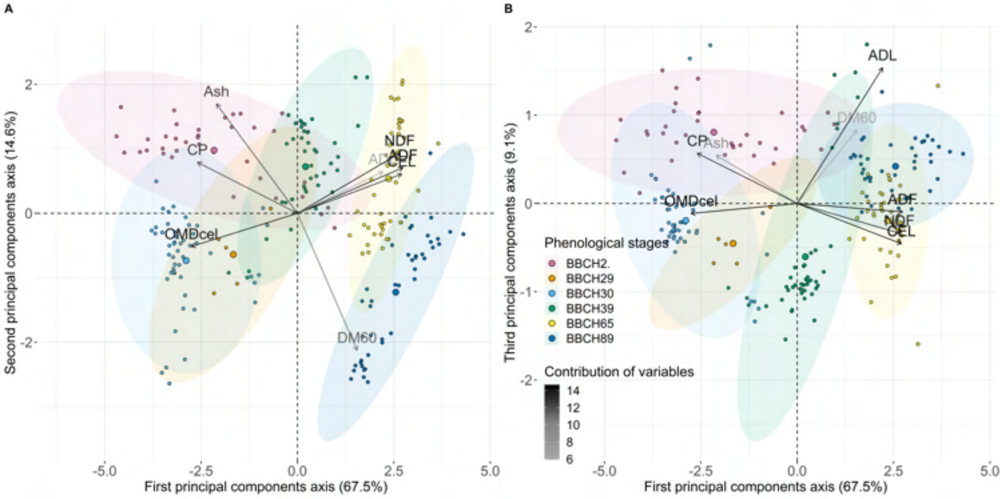

FIGURE 7.2 – Principal component analysis for the consolidated dataset used to develop the NIRS predictive models : first and second principal component axis.

Through the variability mainly induced by phenological stages, the contents expressed in g kg^{-1} of DM obtained by standard chemical analyses ranged from 31 to 133 for Ash, from 28 to 313 for CP, from 345 to 799 for NDF, from 164 to 514 for ADF, from 11 to 112 for ADL and from 144 to 528 for CEL. OMD_{cel} ranged from 28 to 93 g per 100g of DM.

1.3.1.2. NIRS models performances

The 223 generated spectra exhibited spectral features expected for dried forage plant samples (Figure 7.4).

Performances of the PLS models developed for the NIRS prediction (Table 7.3) and the relationship between observed and predicted values for the calibration and validation (Figure 7.3) indicated that the developed models performed quite well. Except for the ADL content, EF was always above 0.95 for calibration and 0.90 for validation, the SEC values were relatively low and close to SEP values, and the RPD values were above 3. Reduced quality of prediction for the ADL content was observed with lower values of EF and RPD (Table 7.3).

TABLE 7.3 – Summary statistics for the calibration and validation of PLS models for the various forage parameters.

		Calibration						Validation					
	FPLS	N	Mean	SD	EF	SEC	RPD	N	Mean	SD	EF	SEP	RPD
Ash	20	156	67.2	21.4	0.95	0.50	4.30	67	66.3	22.6	0.90	0.68	3.27
CP	18	155	112.1	61.4	0.99	0.66	9.51	67	117.5	68.8	0.99	0.75	8.90
NDF	17	155	607.1	119.4	0.99	1.26	9.23	66	591.3	118.0	0.96	2.45	5.12
ADF	17	155	344.8	93.8	0.98	1.27	7.38	66	332.7	96.7	0.98	1.46	6.63
ADL	24	154	47.6	20.5	0.77	1.01	2.07	66	43.8	19.8	0.64	1.11	1.67
CEL	17	152	332.7	89.5	0.98	1.29	6.80	65	318.2	88.9	0.96	1.84	5.10
OMDcel	17	88	57.47	18.61	0.98	2.82	6.57	38	60.55	19.80	0.97	3.20	6.31

Note. : FPLS : number of PLS factors to explain 50 % of variability ; N : number of observations ; Mean : mean of forage parameters, SD : standard deviation of forage parameters ; EF : modelling efficiency ; SEC : standard error of calibration ; SEP : standard error of prediction ; RPD : ratio of standard deviation. Ash, CP, NDF, ADF, ADL and CEL were expressed in g kg^{-1} ofDM and OMD_{cel} in g 100g^{-1} of DM.

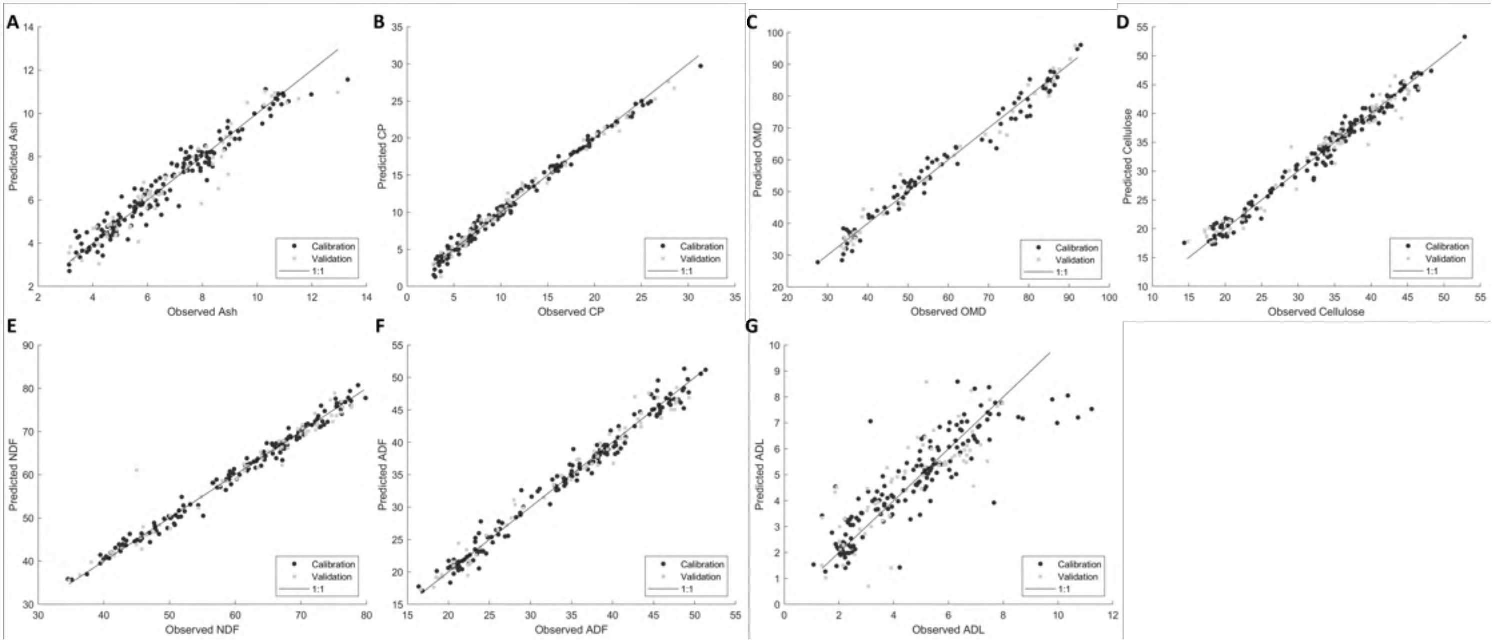

FIGURE 7.3 – Relationship between values measured with the standard reference methods (i.e., observed values) and values predicted by NIRS in the calibration and validation sets for (A) ash, (B) crude protein (CP), (C) enzymatic in vitro digestibility (OMD_{cel}), (D) crude cellulose (CEL), (E) neutral detergent fiber (NDF), (F) acid detergent fiber (ADF) and (G) acid detergent lignin (ADL). The solid line is the relationship between measured and predicted values.

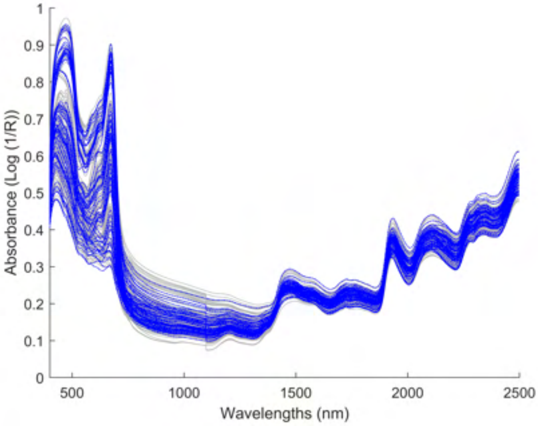

FIGURE 7.4 – The 223 generated pre-processed spectra of the consolidated dataset used to develop the NIRS predictive models, spectra in grey were used for calibration and spectra in blue were used for validation. As highlighted by Deaville and Flinn (2000), this type of spectra has prominent absorption bands including water at 1450 and 1940 nm ; aliphatic carbon - hydrogen bands (lipids) at 1210, 1400, 1725 and 2310 nm ; oxygen - hydrogen bands (carbohydrates) at 1600 and 2100 nm and nitrogen - hydrogen bands (amide structures in protein) at 2055 and 2180 nm.

1.3.2. Forage production of *Th. intermedium* in mixture with legumes

1.3.2.1. Forage quantity

As illustrated in Figure 7.5, an establishment year was observed for both *Th. intermedium* and legumes through an increase of biomass from the first to the second cropping year. Particularly for legumes, this establishment was marked by the increase in their relative importance (in terms of DM) in the mixture over the first growing season (i.e., less than 10 % at BBCH30 compared to 23 % for white clover and more than 40 % for red clover and lucerne at BBCH89).

Globally, biomass production of *Th. intermedium* was low (Figure 7.5). Grown alone, it ranged from 4.8 to 5.8 t of DM ha^{-1} at grain maturity (i.e., BBCH89). In 2023, the spring forage harvest (only performed this year) allowed an additional exportation of biomass of 1.7 t ha^{-1} at the beginning of the reproductive phase (i.e., BBCH30). Low autumn regrowth (i.e., 0.4 t of DM ha^{-1} at BBCH2.) was also observed for *Th. intermedium*, representing lower levels compared to the biomass production at the BBCH30 stage (p-value$<$0.001).

The biomass production at grain maturity and at autumn regrowth was increased (p-value$<$0.01) when a mixture was performed, especially with lucerne and red clover, as they were the two most productive legumes (e.g., red clover reached 4 to 5.2 t of DM ha^{-1} and lucerne reached 3 to 7.1 t of DM ha^{-1}, compared to 0.2 to 1.2 t of DM ha^{-1} for white clover at BBCH89 ; Figure 7.5). At the autumn vegetative stage (i.e., BBCH2.), the legume mixtures increased the biomass production from 225 to 600 % compared to the production of *Th. intermedium* in monoculture. At this stage, *Th. intermedium* was completely dominated by lucerne and red clover (i.e., more than 70 % of legumes within the mixture). Indeed, a strong competition from red clover and lucerne on *Th. intermedium* was observed, especially in 2023, reducing its production (p-value$<$0.001). The relative loss of biomass of *Th. intermedium* in mixture compared to its monoculture at grain maturity was from 0.5 to 4.8 t ha^{-1} when associated to red clover or lucerne and from 0.4 to 1.1 t of DM ha^{-1} when associated to white clover (Figure 7.5). In contrast, white clover was dominated by *Th. intermedium* and represented less than 10 % of the mixture at BBCH89 in 2023.

1.3.2.2. Forage composition and nutritive value

The forage composition of legumes is presented in Table 7.4. Globally, lucerne had the highest content in CEL, NDF and ADF and the lowest in OMD_{cel} and NE_L. The opposite was observed with white clover, and red clover showed intermediate values (p-value$<$0.05). The CP content of legumes didn't vary across species (Table 7.4).

Concerning OM, it ranged from 888 to 966 g kg^{-1} of DM (Figure 7.6A) and was composed from 21 to 31 % of hemicellulose (i.e., NDF minus ADF), from 16 to 40 % of cellulose (i.e.,ADF minus ADL), from 2 to 7 % of lignin (i.e., ADL), from 2 to 31 % of CP and from 2 to 3 % of crude fat for *Th. intermedium* monoculture. The remaining part was represented by the NFC, ranging from 15 to 42 % of the OM. Globally, the OM increased during the growing season (p-value$<$0.001). When an effect of the species mixture treatments was observed, OM was generally lower in mixtures compared to the monoculture (p-value$<$0.01).

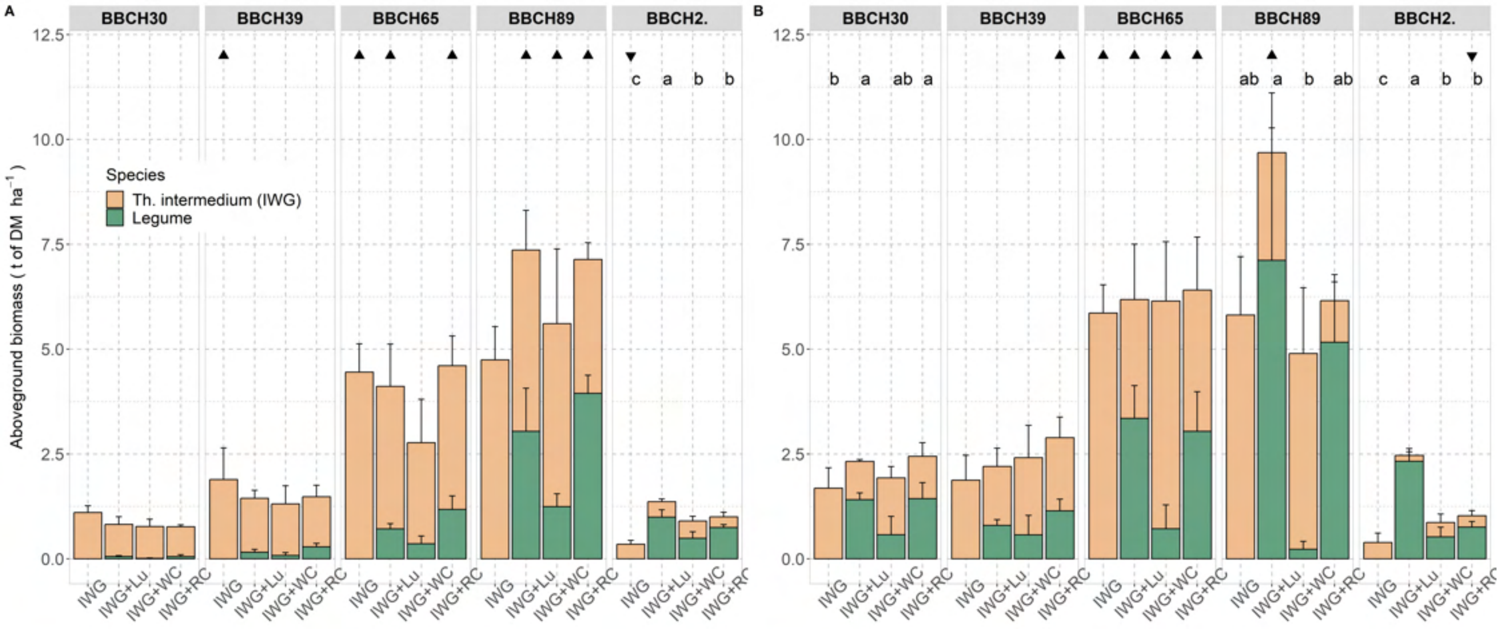

FIGURE 7.5 – Total aboveground biomass of the various species mixture treatments during the growing season of (A) 2022 and (B) 2023. Standard errors are indicated by error bars. Letters represent the results of the post hoc analysis of the effect of the species mixture for each phenological stage (i.e., each letter is assigned to a boxplot representing the species mixture treatment). Bar plot with a symbol indicates the results of the post hoc analysis of the effect of the phenological stage for each species mixture treatment ; ▽ specifying a decreased value ; and △ specifying an increased value compared to the previous phenological stage, except at BBCH2. where the comparison is with the BBCH30 stage. IWG for intermediate wheatgrass - *Th. intermedium*, Lucerne (Lu) for lucerne, WC for white clover and red clover (RC) for red clover.

TABLE 7.4 – Forage qualities of the different legume species during the cropping year of (A) 2022 and (B) 2023. Lu for lucerne, WC for white clover and RC for red clover.

	OM			CP			CEL			NDF			ADF			ADL			OMDcel			NEL		
	WC	RC	Lu	WC	RC	Lu	WC	RC	Lu	WC	RC	Lu	WC	RC	Lu	WC	RC	Lu	WC	RC	Lu	WC	RC	Lu
(A) 2022																								
BBCH30	894±7ab B	905±2c A	905±2bc A	232±7a	245±6a	235±5a	164±7c C	179±6d B	215±9d A	286±8c B	291±6d B	336±7c A	230±9bc B	220±7c B	260±10cd A	39±2bc B	40±1c B	43±2bc A	93±1a A	88±1a B	85±2a C	1827±29a A	1768±15a B	1715±29a C
BBCH39	897±7ab	918±2b	909±2a	180±7b	170±6b	178±5b	184±7bc C	223±6c B	254±9bc A	306±8bc B	326±6c B	387±7b A	249±9bc B	266±7b AB	296±10bc A	42±2ab B	47±1b AB	50±2ab A	87±1bc A	77±1c B	74±2bc B	1691±29b A	1565±15c AB	1521±29b B
BBCH65	909±7ab B	925±2ab A	918±2ab B	164±7bc B	144±6bc C	185±5b A	216±7b C	251±6b B	276±9b A	326±8b C	365±6b B	410±7b A	270±9ab B	288±7b AB	304±10b A	46±2a B	54±1a A	55±2a A	82±1c A	71±1d B	68±2c B	1618±29c A	1461±15d B	1428±29b B
BBCH89	921±7a B	935±2a A	918±2a B	146±7c	129±6c	150±5c	268±7a C	303±6a B	350±9a A	398±8a B	421±6a AB	450±7a A	308±9a B	335±7a AB	368±10a A	44±2ab B	51±1ab A	53±2a A	71±1d A	60±1e B	53±2d B	1457±29d A	1308±15e B	1214±29c B
BBCH2	885±7b	893±2d	896±2c	249±7a	250±6a	256±5a	177±7c B	184±6d B	221±9cd A	274±8c B	285±6d B	334±7c A	226±9c B	214±7c B	252±10d A	35±2c B	38±1c A	39±2c A	93±1ab A	82±1b B	80±2ab B	1815±29ab A	1680±15b B	1649±29a B
(B) 2023																								
BBCH30	887±3c B	902±2b A	890±2c B	248±9a A	220±6b B	248±3a A	201±8b B	201±6b B	246±5c A	301±13b B	313±7b B	352±5c A	258±8b B	235±6c C	291±6b A	40±10c	38±1d	39±1d	88±2a A	81±1ab B	78±1a C	1748±24a A	1638±17ab B	1606±14a B
BBCH39	894±3bc B	904±2b A	888±2c B	207±9b B	207±6b B	231±3b A	208±8b B	208±6b B	262±5bc A	335±13b B	329±7b B	390±5b A	264±8b B	241±6c B	303±6b A	42±10c	42±1c	45±1c	83±2a A	79±1b B	74±1b C	1651±24a A	1600±17b A	1528±14b B
BBCH65	912±3a B	928±2a A	917±2a B	141±9c AB	126±6c B	151±3c A	257±8a B	282±6a B	367±5a A	392±13a B	422±7a B	512±5a A	310±8a B	312±6b B	376±6a A	47±10b	51±1b	53±1b	72±2b A	62±1c B	53±1c C	1460±24b A	1341±17c B	1207±14c C
BBCH89	906±3ab B	921±2a A	915±2a A	162±9c A	139±6c B	135±3d B	282±8a B	308±6a B	372±5a A	400±13a C	446±7a B	528±5a A	326±8a B	341±6a B	402±6a A	52±10a C	57±1a B	62±1a A	67±2b A	55±1d B	43±1d C	1392±24b A	1232±17d B	1068±14d C
BBCH2.	888±3c B	904±2b A	902±2b AB	242±9a	249±6a	220±3b	200±8b B	190±6b B	273±5b A	299±13b B	303±7b B	363±5c A	257±8b B	215±6c C	301±6b A	38±10c B	36±1d B	42±1cd A	87±2a A	83±1a B	75±1ab C	1725±24a A	1703±17a A	1557±14ab B

Note. : enzymatic in vitro digestibility (OMD_{cel}) is expressed in g per 100 g of DM, organic matter (OM), crude protein (CP), crude cellulose (CEL), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) in g per kg of DM and net energy for lactation (NE_L) in kcal per kg of DM.

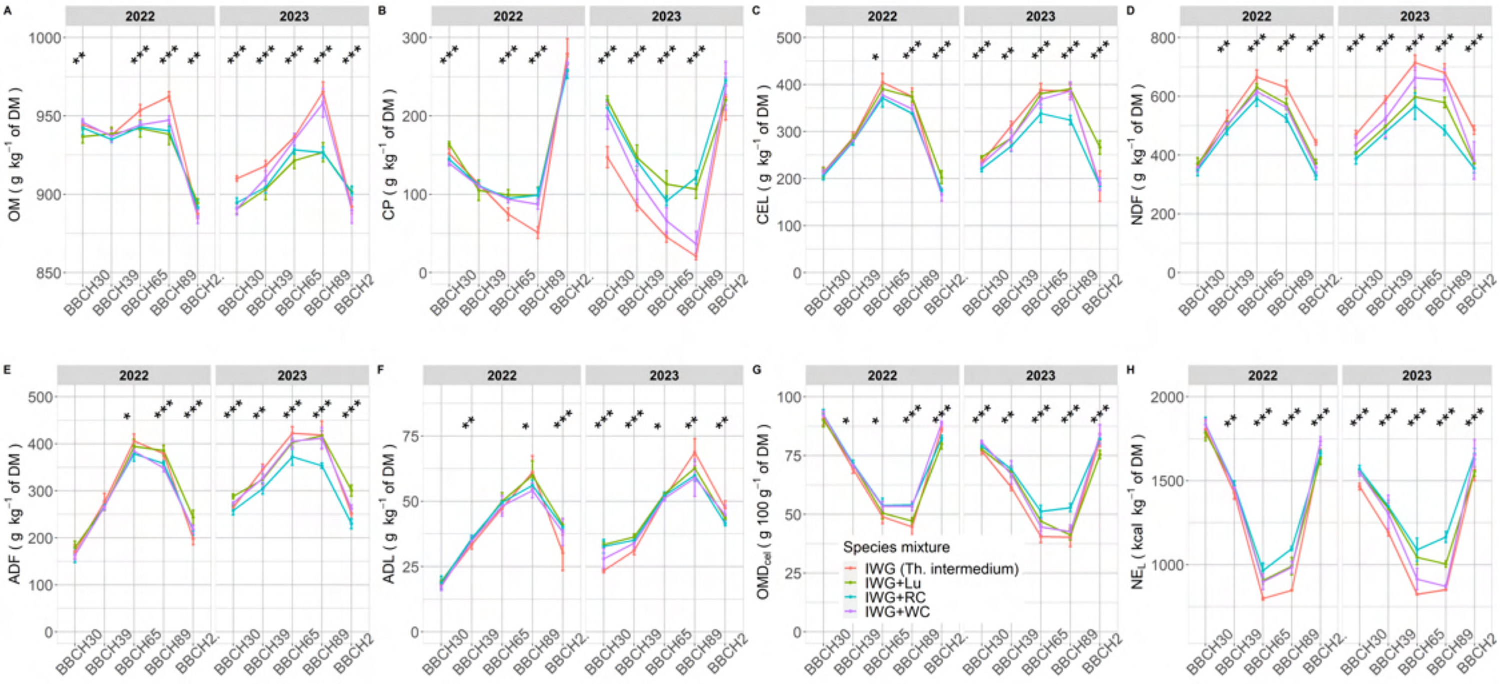

FIGURE 7.6 – Evolution of the forage parameters (A) organic matter (OM), (B) crude protein (CP), (C) crude cellulose (CEL), (D) neutral detergent fiber (NDF), (E) acid detergent fiber (ADF), (F) acid detergent lignin (ADL), (G) enzymatic in vitro digestibility of OM (OMD_{cel}), (H) net energy for lactation NE_L of the different species mixture treatments during the growing season of 2022 and 2023. Statistical differences (post hoc analysis) between species mixture treatment for each phenological stage are indicated by '*' with a statistical significance at p-value<0.05, '**' with a statistical significance at p-value<0.01, '***' with a statistical significance at p-value<0.001. IWG for intermediate wheatgrass - *Th. intermedium*, Lu for lucerne, WC for white clover and RC for red clover.

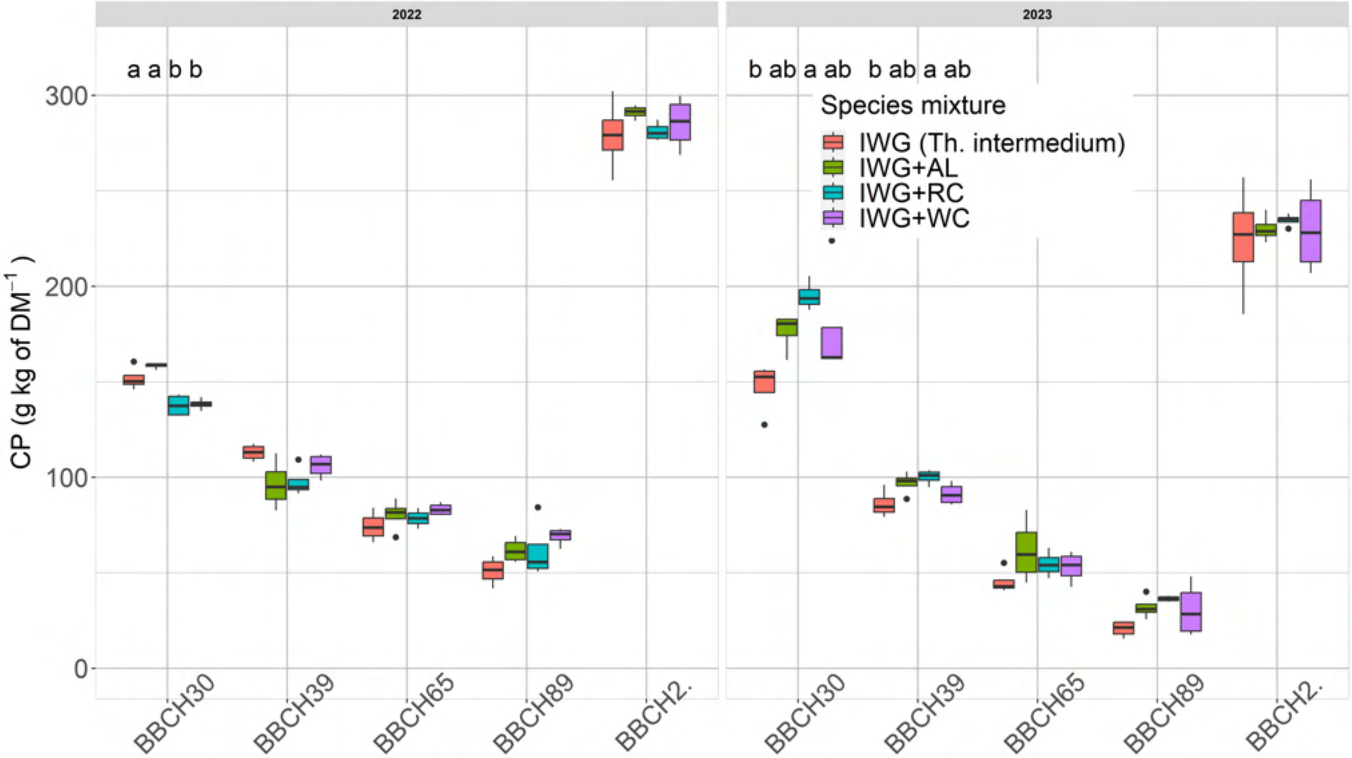

FIGURE 7.7 – Evolution of crude protein (CP) of *Th. intermedium* pure forage under various species mixture treatments during the growing season of 2022 and 2023. Letters represent the results of the post hoc analysis of the effect of the species mixture for each phenological stage (i.e., each letter is assigned to a boxplot representing the species mixture treatment at each phenological stages), with a p-value<0.001 at BBCH30 of 2022 and p-value<0.01 at BBCH30 and BBCH39 of 2023. IWG for *Th. intermedium*, Lu for Lucerne, WC for white clover and RC for red clover.

As shown by the PCA (Figure 7.2), the forage quality of the different mixture treatments was influenced by the phenological stages. Fiber content (i.e., CEL, NDF, ADF and ADL) increased during the growing season, while CP, OMD_{cel} and NE_L decreased (p-value<0.001 ; Figure 7.6). For the monoculture of *Th. intermedium*, the highest CEL, NDF and ADF contents were observed at the flowering stage (i.e., BBCH65 ; mean, in g kg^{-1} of DM, of 396 for CEL, 690 for NDF and 415 for ADF). The highest content of ADL was observed at the grain maturity stage (i.e., BBCH89 ; mean of 65 g kg^{-1} of DM). Concerning CP, OMD_{cel} and NE_L the highest values were observed at vegetative stages (i.e., BBCH30 and BBCH2.) with a mean of 201 g kg^{-1} of DM, 84 g per 100 g of DM and 1625 kcal kg^{-1} of DM, respectively.

The forage quality was modified by legumes once they were well established (i.e., representing roughly 20 % of the mixture, Figures 7.5 and 7.6) at a leafy vegetative stage, as early phenological stage induced the best forage quality (Table 7.4). Thus, red clover had globally the major impact on the forage quality compared to white clover and lucerne (Figure 7.6). In mixture, the NDF content was reduced by about 87 g kg^{-1} of DM regardless of the legume species (p-value<0.01). At late phenological stages (i.e., BBCH65 and BBCH89), CEL and ADF decreased in the red clover mixture by about 43 g kg^{-1} of DM (p-value<0.05). Focusing on the CP content, the mixture with legumes buffered the decrease over the growing season, especially with red clover and lucerne (p-value<0.001) as no effect was observed at the autumn vegetative stage (i.e., BBCH2.). Concerning the OMD_{cel}, the highest increase was observed at late phenological stages with the red clover mixture (e.g., maximal increase of 13 g per 100 g of DM). Finally, legumes increased the energy value NE_L and particularly the red clover mixture (e.g., maximal increase of 313 kcal kg^{-1} of DM). Focusing on *Th. intermedium* forage parameters in the mixture, the CP content was the only parameters influenced by legumes at the beginning of the second year (i.e., BBCH30 and BBCH39), with a maximal increase of 30 g kg^{-1} of DM with red clover at BBCH30 (Figure 7.7).

1.3.3. Grain production of *Th. intermedium* in mixture with legumes

Overall, *Th. intermedium* grain yields were low (Figure 7.8) and ranged from 345 to 616 kg ha^{-1}. This was partly explained by a weak establishment of the crop during the two first years with an average tiller density of 260 tillers m^{-2} during the entire first reproductive phase and 690 tillers m^{-2} at the beginning of the second reproductive phase, regardless of the species mixture treatment. This led to low spike densities in the first year (i.e., ranging from 165 to 257 spikes m^{-2}). In addition, the spring forage harvest performed in the second year reduced the grain yield potential by about half compared to no spring forage harvest (data not shown).

As observed in Figure 7.8, the mixture with a legume impacted the grain yield ($p\text{-value}<0.05$) with the highest grain yield obtained with *Th. intermedium* monoculture and the lowest with the mixture of *Th. intermedium* with lucerne and red clover. Furthermore, in the second year, the mixtures with lucerne and red clover led to the impossibility of harvesting grains due to lodging. Spike density was also impacted by the mixture with legume ($p\text{-value}<0.01$), with the highest densities obtained with the monoculture (i.e., 260 and 440 spikes m^{-2} in the first and second year, respectively) and the lowest with *Th. intermedium* associated with red clover (i.e., 165 and 56 spike m^{-2} in the first and second year, respectively).

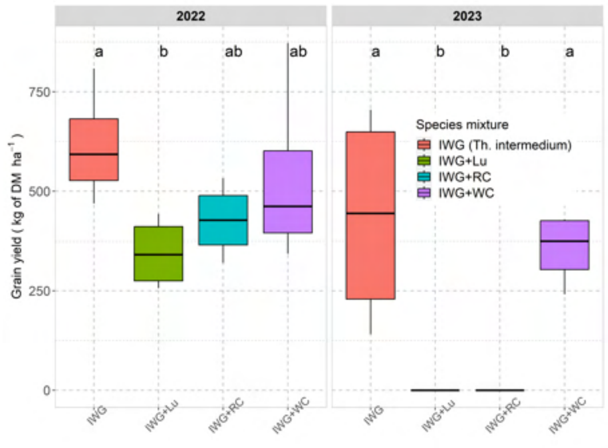

FIGURE 7.8 – Grain yield of *Th. intermedium* under various species mixture treatments in 2022 and 2023. Statistical differences (post hoc analysis) between species mixture treatment are indicated by letters (i.e., each letter is assigned to a boxplot representing the species mixture treatment), with a $p\text{-value}<0.05$ in 2022 and a $p\text{-value}<0.01$ in 2023. IWG for intermediate wheatgrass - *Th. intermedium*, Lu for lucerne, WC for white clover and RC for red clover.

1.4. Discussion

1.4.1. NIR spectrometry prediction

Th. intermedium samples used to develop the NIR models showed a wide range of values for the different forage parameters. Although the database contained various cropping sites, this variability mainly reflected the ageing tissues from different phenological stages. This was also observed in Culman et al. (2023) where the forage nutritive value was primarily driven by seasonal trends and to a much lesser extent by stand ages. Good quality predictions were obtained for most forage parameters through the different quality criteria. An exception can be mentioned with ADL with a lower quality of prediction due to the weak repeatability of the chemical analysis (Table 7.3). As our NIR calibration was based on a single species, the predictions were fairly accurate, but could involve less robustness (Deaville and Flinn, 2000). Although satisfactory, this initial model should be further completed with additional data coming from new climatic years to broaden the database. Nevertheless, this first calibration offered a fast and efficient tool to characterize the forage composition of *Th. intermedium* across various pedoclimatic conditions and management practices. In addition, as present-day breeding efforts on *Th. intermedium* for grain yield improvements could constrain the production of vegetative tillers to the benefit of reproductive ones (Altendorf et al., 2021), this method could be helpful to assess the effect of breeding advances on forage quality.

1.4.2. Forage production potential of *Th. intermedium*

The different forage parameters of *Th. intermedium* were in agreement with other studies on the species (Table 7.5). Forage quality of *Th. intermedium* at vegetative stages (i.e., BBCH30 and BBCH2.) was satisfactory with an NE_L of 1625 kcal kg^{-1} of DM (Figure 7.6), which was close to a fresh grass (Table 7.5). Indeed, the various forage parameters of *Th. intermedium* were close to those of common pasture grasses such as *Phleum pratense* L., *Lolium perenne* L., *Festuca pratensis* Huds., *Festuca arundinacea* Schreb. and *Dactylis glomerata* L. at the same stage (Table 7.5). The CP was more variable ranging from 147 to 279 g kg^{-1} of DM and tended to be higher in autumn. Consequently, this fodder could be used to feed lactating dairy cattle with an eventual proper supplementation for balancing the amount of protein and energy in the diet (Cuvelier et al., 2021). However, the biomass production of *Th. intermedium* at vegetative stages was weak, averaging 1.4 t ha^{-1} at the beginning of the spring (i.e., BBCH30) and 0.4 t ha^{-1} at the autumn regrowth. Although our spring production was close to other studies, the autumn production was lower, generally between 1 and 2 t ha^{-1} (Culman et al., 2023; Hunter et al., 2020b). The mean total production of vegetative biomass averaged 1.8 t ha^{-1} over a growing season (Figure 7.5). Pugliese (2017) reported that the production in spring and autumn can, both, reach 4 t ha^{-1}, but only one of the two forage harvests was performed over the growing season.

TABLE 7.5 – Forage parameters of common pastures grasses and other feedstuffs compared to *Th. intermedium* at different phenological stages found in the literature.

	Vegetative stages						Maturity stages						References
	OMD-cel	CP	CEL	NDF	ADF	NEL	OMD-cel	CP	CEL	NDF	ADF	NEL	
Dactylis glomerata L.	78	245	177	490	206		57	95	353	680	393		(INRA, 2018)
Festuca pratensis Huds.	82	235	186	499	215		65	113	342	663	367		(INRA, 2018)
Lolium perenne L.	82	223	197	482	221		60	97	328	629	356		(INRA, 2018)
Phleum pratense L.	79	202	224	500	257		51	72	361	664	375		(INRA, 2018)
Festuca arundinacea Schreb.	74	204	235	546	261		57	10	33	65	36		(INRA, 2018)
Fresh grass	84	219				1665							(CVB, 2022)
Hay of “poor quality”							63	106				1152	(CVB, 2022)
Grass seed straw							55	62				990	(CVB, 2022)
Cereal straw							45	35	420	785	493	812	(CVB, 2022; INRA, 2018)
Th. intermedium		[125-225]		[456-590]	[249-337]			[41-73]		[672-828]	[382-501]		(Barriball, 2020; Culman et al., 2023; Favre et al., 2019; Pinto et al., 2022)

Note. : enzymatic in vitro digestibility (OMD_{cel}) is expressed in g per 100 g of DM, crude protein (CP), crude cellulose (CEL), neutral detergent fiber (NDF) and acid detergent fiber (ADF) in g per kg of DM and net energy for lactation (NE_L) in kcal per kg of DM.

At grain maturity, the quality of *Th. intermedium* forage was relatively low with NE_L averaging 849 kcal kg^{-1} of DM (Figure 7.6). The fiber content of *Th. intermedium* was quite close to the reference values of the different common pasture grasses previously mentioned at a flowering stage. However, the OMD_{cel}, CP and NE_L values of *Th. intermedium* were close to common cereals straw (i.e., wheat, barley and oat ; Table 7.5). As already mentioned in the study of Favre et al. (2019), this crop residues could replace straw in high-starch dairy diets to maintain proper rumen function and prevent acidosis (Hurdebise et al., 2023). The biomass production at grain harvest averaged 5.3 t ha^{-1} (Figure 7.5). This was relatively low compared to the yield potential of *Th. intermedium* in our pedoclimatic conditions that ranged from 7 to 16 t ha^{-1} (Fagnant et al., 2023). It can be explained by the poor establishment of the crop in the first year (i.e., only 260 tillers m^{-2} during the first year) and the spring forage harvest performed in the second year. As observed by Culman et al. (2023), the summer yield potential was highly variable ranging from 2 to 11 t of DM ha^{-1}.

Over a growing season, the yield potential of *Th. intermedium* ranged from 5.2 to 7.9 t ha^{-1} (Figure 7.5). As observed by Pugliese (2017), when a spring, an autumn or both forage harvests are performed, this yield potential varied widely, but was generally exceeding 9 t ha^{-1} (Fagnant et al., 2024; Hunter et al., 2020b). While the biomass production of *Th. intermedium* could reach that of sowed grasslands (i.e., sown European grassland range from 5 to 12 t of DM ha^{-1} y^{-1} and up to 20 t ha^{-1} y^{-1} ; Wilkins (2000)), its forage potential was limited. Indeed, most of the biomass was obtained at grain maturity (i.e., more than 70 % of the biomass of the year) with a forage quality comparable to cereal straw, which was of little value in animal feed. In contrast, only 2 to 4 t of DM ha^{-1}, in best cases, could be valorized as good quality *Th. intermedium* fodder. Spring forage harvest represented a way to increase the proportion of good quality fodder, as it decreased the proportion of biomass harvested at grain maturity from 95 to 73 % and converted the remaining percentage into valuable fodder. However, a trade-off between grain and forage harvest was observed. The spring forage harvest decreased grain yield of *Th. intermedium* in our second year (data not shown), as also observed in other studies (Culman et al., 2023; Hopkins et al., 2003; Zimbric et al., 2021). This could be explained by the removal of the leaf area essential for grain production and the limited accumulation of reserves after the spring harvest (Culman et al., 2023). In addition, *Th. intermedium* regrowth was not sufficient to justify an autumn forage harvest. All these insights highlighted the complexity to produce sufficient high-quality fodder in a growing season dedicated to grain production, suggesting a potential forage valorization within extensive livestock production with moderate production goals. As mentioned by Duchene et al. (2021), *Th. intermedium*, as a slow-growing species, could be more suited to harsh pedoclimatic conditions (e.g., fields at high altitudes or with low resource-availability) through its capacity to produce high levels of biomass with low resources requirements such as nitrogen (Fagnant et al., 2023) and potentially water (Clément et al., 2022; de Oliveira et al., 2020).

1.4.3. Production potential of *Th. intermedium* grown in mixture

The mixture of *Th. intermedium* with legumes could improve the forage potential through quantity and, to a lesser extent, quality. We observed this positive impact when legumes represented at least 20 % of the mixture. As observed in Figure 7.5, this proportion was not encountered before the flowering stage of the establishment year. In addition, we observed differences between legumes, with lower levels of biomass of white clover which was dominated within the mixture contrarily to red clover and lucerne. We also noticed better forage qualities of red clover and white clover compared to lucerne. Through its high biomass production and its good forage quality, red clover had the major impact on the forage quality of the mixture (Figure 7.5; Table 7.4). The positive effect of the legume mixture was mainly observed at late phenological stages, with a reduction of the fiber content (i.e., CEL, NDF, ADF and the increase of the CP, OMD_{cel} and NE_L (Figure 7.6). However, at grain maturity, the production of *Th. intermedium* in mixture with legume resulted in a forage with still little value in animal feed. Depending on the forage parameter compared and the legume used within the mixture, the forage was comparable to common pasture grasses at a flowering stage or grass seed and cereal straws that had lower forage nutritive value than a hay characterized by ‘poor quality’ (Table 7.5). Concerning the forage quantity, at grain harvest, it was increased with the lucerne and red clover mixtures (i.e., mean increase of 2.3 t of DM ha^{-1}; Figure 7.5). In autumn, due to the lack of regrowth of *Th. intermedium*, a significant increase of the forage quantity was observed for all the legume mixtures (i.e., mean increase of 1 t ha^{-1}; Figure 7.5). In the study of Favre et al. (2019) red clover mixture increased forage yield around 3 t ha^{-1} over the year and its CP content as it decreased fiber content in autumn.

The increase of the total biomass production when *Th. intermedium* was associated to red clover or lucerne came at the expense of *Th. intermedium* growth. The crop showed little competitiveness over these two species with a loss of biomass compared to its monoculture at BBCH89 from 1 to 5 t ha^{-1} (Figure 7.5). This was also reflected in grain yield component with a loss from

22 to 82 % of spike density and therefore a reduction of the grain yield from 37 to 100 % (Figure 7.8). Indeed, the strong competition of these productive forage legumes (i.e., production level always above 4 t of DM ha^{-1} ; Figure 7.5) induced an impossibility of grain harvesting due to lodging at grain maturity. It was also highlighted by Tautges et al. (2018), where a reduction of the grain yield was observed when *Th. intermedium* was grown in mixture with lucerne that produced from 2 to 4 t of DM ha^{-1}. As Pinto et al. (2022) observed that the high level of red clover and lucerne biomass compromised the establishment of *Th. intermedium* and its grain and forage production. On the contrary, through its low production (i.e., maximum of 1.2 t of ha^{-1} observed at BBCH89 in 2022 ; Figure 7.5), white clover had little effect on grain yield with similar spike density and limited reduction of the grain yield compared to *Th. intermedium* monoculture (Figure 7.8). In the study of Dick et al. (2018), the mixtures with white clover and lucerne didn’t impact the production of *Th. intermedium* since their production of biomass didn’t exceed 1 t ha^{-1}. Pinto et al. (2022) suggested that the early *Th. intermedium* biomass accumulation in the establishment year was essential with aggressive legume’s establishment such as red clover and lucerne. Thus, new agroecosystems should be designed to optimize the complementarity and stability of the mixture of *Th. intermedium* with legume under a dual-use management. Some research was performed to understand how to regulate the competition between *Th. intermedium* and perennial legumes with agronomic management. This included forage mowing in the interrow (Crews et al., 2022), legume frost seeded in the spring on *Th. intermedium* crop planted in the previous autumn (Olugbenle et al., 2021; Pinto et al., 2022) or the implantation of annual legume such as berseem clover (Pinto et al., 2022) to reduce competition. As suggested by Culman et al. (2023), management of the crop could also be shifted from grain production to a single-purpose forage production over the cropping years, enabled by the stability of the forage quantity and quality of *Th. intermedium* over time. Finally, the implantation of legumes may take place after the first years of *Th. intermedium* grain production to allow proper establishment of the crop and maximize the benefits of the legume mixtures for forage production.

1.5. Conclusions

Through proper model calibrations, near-infrared spectrometry offered an efficient and easy-to-use tool to predict the forage chemical composition and enzymatic in vitro digestibility of *Th. intermedium*, with the need to continuously supply the database to catch the maximal variability of forage constituents. *Th. intermedium* forage potential was reduced as most of the biomass harvested in a dual-use perspective had poor nutritional value. The intensification of forage production through the spring forage harvest or the mixture with competitive legumes came at the expense of the grain production of *Th. intermedium*. Therefore, in the perspective of a dual-use management, the implantation of companion legume such as white clover, in case of good stand establishment, could slightly enhance the forage yield potential (i.e., increase of nutritive value and of forage quantity at autumn regrowth) without hampering the grain production. More competitive legumes, like red clover and lucerne, require more work to find the best varieties or innovative management options in fields. All these insights can inform the on-going process of *Th. intermedium*'s breeding and help farmers to design relevant systems to experiment this new crop.

2. Production fourragère sous une gestion simple usage

Introduction au chapitre

Cette deuxième section du chapitre 7 n’est actuellement pas destinée à être publiée scientifiquement.

Cette section vise a étudier le potentiel de *Th. intermedium* sous une gestion simple usage uniquement fourragère. Pour ce faire, lors d’une seule année culturale, de multiples fauches ont été réalisées pendant la saison de croissance pour quantifier la quantité et la qualité de la biomasse produite par rapport à des gestions à double usage grain-fourrage. Les données récoltées apportent une réelle contribution à la caractérisation des perfomances fourragères de l’espèce. En effet, aucune nouvelle référence n’existe pour ce type de gestion sur la nouvelle génétique de *Th. intermedium* en tant que culture céréalière pérenne.

Credit authorship contribution statement

Laura Fagnant : conceptualization, planning of the experiments, formulation of research goals and aims, development and design of methodology, carrying out the samplings, data curation, formal analyses (statistical and mathematical), interpretation of result, data presentation and visualization, writing – original draft preparation.
Lucas Villé : carrying out the samplings.

2.1. Introduction

Through breeding for higher yields and large seeds, *Thinopyrum intermedium* is now proposed as a dual-use crop with income provided through both forage and grain (Hunter et al., 2020b). Originally, the plant was introduced into North America from Eurasia in 1932 as a forage crop for erosion control, revegetation, and forage production (Barkworth et al., 2007; Hunter et al., 2020b). Early studies suggested that *Th. intermedium* could be used for haying (Lawrence and Ashford, 1966) or spring and autumn grazing (Moore et al., 1995), that was widely performed in the Northern and Central Great Plains region of the USA (Hendrickson et al., 2005). Indeed, *Th. intermedium* can represent an interesting forage source for dryland agriculture such as in the Great Plains in North America, the Canadian prairie region or in New Zealand (Heinrichs and Clark, 1961; Moore et al., 1995; Wills et al., 1998). Under less intensive use, *Th. intermedium* had a higher yielding ability as a hay grass than other grasses (Heinrichs and Clark, 1961). Lawrence and Ashford (1966) harvested more than 11 t ha^{-1} of biomass in a single season with repeated defoliation. However, other past studies indicated that *Th. intermedium* is not well-suited for use in pastures. Under repeated frequent defoliations, stand vigor declined and susceptibility to winterkill increased. Indeed, Campbell (1961) suggested that *Th. intermedium* is not recommended for long-term pastures as the continuous grazing treatment predisposed its disappearance. Heinrichs and Clark (1961) observed that *Th. intermedium* halves its biomass production under frequent defoliations compared to a unique forage harvest at flowering. The adverse effect of frequent defoliations was also highlighted by Lawrence and Ashford (1966) that indicated that the recovery period between defoliations and the time of forage utilization is essential to maintain the stand and the forage yields.

Currently, improved lines of *Th. intermedium* are used in a dual-use perspective for grain production. Its seeds are marketed under the trade name Kernza® in North America (Soto-Gómez and Pérez-Rodríguez, 2022). Recent studies identified some limitations of economic viability of the crop through the low initial grain yield and its decline with stand age (Culman et al., 2023; Hunter et al., 2020a). As suggested by Culman et al. (2023), most of its net primary productivity is directed to non-reproductive structures. Dual-use management strategies, valuing forage in addition to grain could represent a potential viable way to increase to productivity and therefore the profitability of the crop. Recent studies have effectively demonstrated its capacity to produce high levels of biomass with low resources requirements such as nitrogen (Fagnant et al., 2023). However, dual-use strategies with spring or autumn mowing in addition to grain harvest, could constrain grain yield production (Clément et al., 2022; Zimbric et al., 2021). In this way, the dual-use management could be transformed into a unique forage use management with aging crop (Puka-Beals et al., 2022), enabled by the stability of forage quantity and quality of *Th. intermedium* over time (Culman et al., 2023). In addition, intercropping legumes with a perennial grass such as *Th. intermedium* can provide multiple benefits such as increasing the total forage harvested and its nutritional value (Favre et al., 2019; Pinto et al., 2022).

Since it is essential to find new ways of forage valorization of *Th. intermedium*, the aim of this study was therefore to assess its forage production under a unique forage use management. To do so, the crop was grown in monoculture or in mixture with different legume species and subjected to multiple forage harvests during the growing season. The forage production on a unique forage use management was compared to other dual-use managements during a single cropping year.

2.2. Materials and methods

2.2.1. Experimental site

Data collected on the BE3 experimental site in Belgium (Tables 7.1 and 7.2) was used to characterize the forage production of *Th. intermedium* in terms of quantities and qualities under a simple-use management compared to the two different dual-use managements. As previously explained, this field experiment was conducted during two successive cropping years using a complete randomized split-plot design (4 x 8 m subplots) with a forage harvest factor as the main-plot treatment and a species mixture factor as the split-plot treatment, with four replicates. The forage harvest factor compared different treatments : a unique forage use management where multiple cuts were performed during the growing season (i.e., multiple cuts treatment) ; a dual-use management where a spring cut was coupled with the grain harvest (i.e., spring cut treatment) ; and a dual-use management where only the grain harvest was performed during the growing season (i.e., grain harvest treatment ; Table 7.2). Three successive cuts were applied for the multiple cuts treatment : May 5, 2023, corresponding to the BBCH30 stage, May 30, 2023, corresponding to the BBCH39 stage of the dual-use treatments and June 28, 2023, corresponding to the BBCH65 stage of the dual-use treatments. This forage harvest factor could only be compared in the second cropping year as spring cuts were only performed in the second year due to insufficient plant establishment during the first year.

An autumn cut was performed in the establishment year for each treatment and the field management is described in Table 7.1. During the mechanical forage harvests, the aboveground biomass was cut at 7 cm above the soil surface (i.e., above the apex height) and exported from the field. In this experiment, four treatments compared the effects of the mixture with different legumes species : I) *Th. intermedium* monoculture (IWG), II) *Th. intermedium* in mixture with white clover (i.e., *Trifolium repens* L.), III) *Th. intermedium* in mixture with red clover (i.e., *Trifolium pratense* L.) and IV) *Th. intermedium* in mixture with lucerne (i.e., *Medicago sativa* L.). Legumes were seeded in the interrow of *Th. intermedium*, but only on the half of all interrow to reduce light competition as described in Figure 7.1.

2.2.2. Data collection

Aboveground biomasses were sampled during the growing season with a 50 x 50 cm quadrat, cut at 5 cm above the soil surface, fresh samples were weighted to obtain the fresh matter and then oven-dried (72 h at 60 °C) and weighted again to obtain the dry matter. Samples were collected at different phenological stages, rated with the BBCH scale (Meier, 2018), from the beginning of stem elongation (BBCH30) to the grain maturity (BBCH89) stage as illustrated in Figure 4.7. The cumulated production of biomass over the growing season was calculated by summing the biomass production of the different cuts to the final production at BBCH89. As lodging was observed on the mixture of *Th. intermedium* with lucerne and red clover of the grain harvest treatment, the final production was estimated with the production at the flowering stage (i.e., BBCH65).

Samples were then ground to a particle size of 1 mm in a FOSS Tecator Cyclotec 1093 mill ; Foss company ; Hillerød ; Denmark. The dried and ground samples were subjected to near-infrared radiation with wavelengths ranging from 400 to 2498 nm by using a XDS Monochromator Type XM-1000 FOSS spectrometer ; Foss company ; Hillerød ; Denmark. The spectral data were recorded with a step of 2 nm. The spectral absorbance values were recorded as log (1 $R^{-}1$), where R is the sample reflectance. Spectral data were used to predict the forage chemical composition and enzymatic in vitro digestibility of *Th. intermedium* derived from the predictive model described in the previous section 1.2.3. from chapter 7.1. From the predicted forage parameters, the NE_L of *Th. intermedium* was calculated according to the Dutch feed evaluation system for ruminants (CVB, 1991). According to the forage composition of samples (i.e., ash, fiber content, digestibility or crude protein) various feed equations were used to estimate NE_L values. For the dual-use managements, the 'fresh grass' equations was used for the vegetative stages (i.e., BBCH30) ; 'hay' equations for the BBCH39 stage and 'straw' equations for the BBCH65 and BBCH89 stages. Concerning the unique forage use management, the 'fresh grass' equations was used for the vegetative stages (i.e., BBCH30) representing the first cut and the 'hay' equation for the two other successive cuts corresponding to the BBCH39 and BBCH65 stage of the dual-use managements. The forage parameters of the third cut will not be discussed in the following sections as the biomass production at that time was almost non-existent.

2.2.3. Data analysis

Data analyses was conducted in the R program version 4.1.2 (R Core Team, 2024). A three-way ANOVA was performed with mixed model applied with *lmer* function from the *lme4* package. The species mixture factor, the forage harvest factor and the phenological stage were considered as fixed effect, while replicates as random effect. Models were evaluated to ensure they met the assumptions of independence and normality of residuals through the *plotresid* function. Following the ANOVA, 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. Aboveground biomass of *Th. intermedium* and legumes were the analyzed variables.

2.3. Results and discussion

2.3.1. Forage production under various legume mixture

The aboveground biomass produced during the growing season is presented in Figure 7.9. For the spring cut treatment one exportation of biomass was performed at the BBCH30 stage (i.e., represented by the biomass production at this stage ; Figure 7.9B). Concerning the multiple cuts treatment, exportations were performed at the BBCH30, BBCH39 and BBCH65 stages (i.e., represented by the biomass production at these stages ; Figure 7.9C). Considering the cumulated production of biomass over the growing season is therefore interesting (i.e., sum of the biomass production of the different cuts and the final production at BBCH89) to compare treatments.

Performing multiple cuts was positive for the production of white clover by increasing its cumulated biomass ($p\text{-value}<0.001$). The opposite was observed for red clover with the highest biomass observed with the grain harvest treatment ($p\text{-value}<0.05$). Lucerne production wasn't impacted by the forage harvest factor (Figure 7.9).

Forage productivity (i.e., in terms of quality and quantity) of the different legumes within the mixture as well as their effect on *Th. intermedium* are described in the section 1.3.2. from chapter 7.1. Within the different forage harvest treatments, the same trend was observed with the highest total final biomass production (i.e., at BBCH89) obtained with the mixture with red clover and lucerne ($p\text{-value}<0.001$). However, for the grain harvest and the spring cut treatments, severe lodging was observed at the end of the growing season for the plots sown under these mixtures (Figure 7.10D). Which prevented the harvest of the plots subjected to the grain harvest treatment at the end of the growing season (Figure 7.9A). Indeed, a strong competition was induced by these two productive legumes as previously discussed.

2.3.2. Forage production under various forage harvests

Focusing on *Th. intermedium*, the cumulated production of its monoculture was similar between the spring cut and the grain harvest treatments, averaging 7.5 and 7.6 t of DM ha^{-1}, respectively. As also observed by Pugliese (2017), the spring forage cut didn't decrease the total biomass production of *Th. intermedium* monoculture over the growing season. Concerning the forage parameters of *Th. intermedium* during the growing season, they were similar between these two treatments ($p\text{-value}>0.05$) and are presented in Figure 7.6 for the spring cut treatment. Indeed, performed above the apical mersitems, the spring cut didn't modify the phenological development of *Th. intermedium*. In addition, it allowed an exportation of 1.7 t of DM ha^{-1} (Figure 7.9) of good quality fodder (i.e., high nutritional values, comparable to a fresh grass; Tables 7.5 and 7.6). However, this spring cut has reduced the grain yield of the crop (section 1.3.3. of chapter 7.1), reducing the potential of a dual-use management. In a unique forage use perspective, the valorization of good quality fodder through spring cut at the beginning of stem elongation could be interesting in the case of harvesting costs offset by forage yields.

Concerning the multiple cuts treatment, the forage parameters estimated at the two first cuts are presented in Table 7.6. As observed with the spring cut treatment, the first cut didn’t modify the phenological development of *Th. intermedium*. Thus, the two first biomass exportations (i.e., BBCH30 and BBCH39 ; Figure 7.9C) had similar forage parameters between the different forage harvest treatments (Table 7.6 and fig. 7.6). The first cut of *Th. intermedium* had similar forage parameters as a crop at the BBCH30 stage (i.e., high nutritional values, comparable to a fresh grass) and second cuts had similar forage parameters as a crop at the BBCH39 stage (i.e., similar to a hay of poor quality ; Tables 7.5 and 7.6). After the second cut, however, the biomass production of *Th. intermedium* at the time of the third cut was extremely weak (Figure 7.9C at BBCH65 ; representing the forage production at the third cut). The regrowth of the stand was almost inexistent, as illustrated in Figure 7.10C. This resulted in a reduced cumulated biomass of *Th. intermedium* monoculture when multiple forage cuts were performed during the growing season, with a mean of 3.9 t of DM ha^{-1}. This represented a loss of 3.7 t of DM ha^{-1} compared to the dual-use managements ($p\text{-value}<0.001$; Figure 7.9).

TABLE 7.6 – Forage parameters of the cuts of the spring cut and the multiple cuts treatments. For the spring cut and the multiple cuts treatments, May 5 - BBCH30 corresponds to the first cut. For the multiple cuts treatment, May 30 - BBCH39 corresponds to the second cut.

	CP	CEL	NDF	ADF	ADL	OMDcel	NEL
May, 5 -BBCH30	182 ± 5	255 ±5	519 ± 5	262 ±4	22 ±2	785 ± 7	1524 ± 10
May, 30 - BBCH39	98 ± 3	347 ± 5	617 ± 6	325±5	32 ± 1	654 ± 9	1281 ± 17

Note. : crude protein (CP), crude cellulose (CEL), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) are expressed in g per kg of DM, enzymatic in vitro digestibility (OMD_{cel}) in g per 100 g of DM and net energy for lactation (NE_L) in kcal per kg of DM.

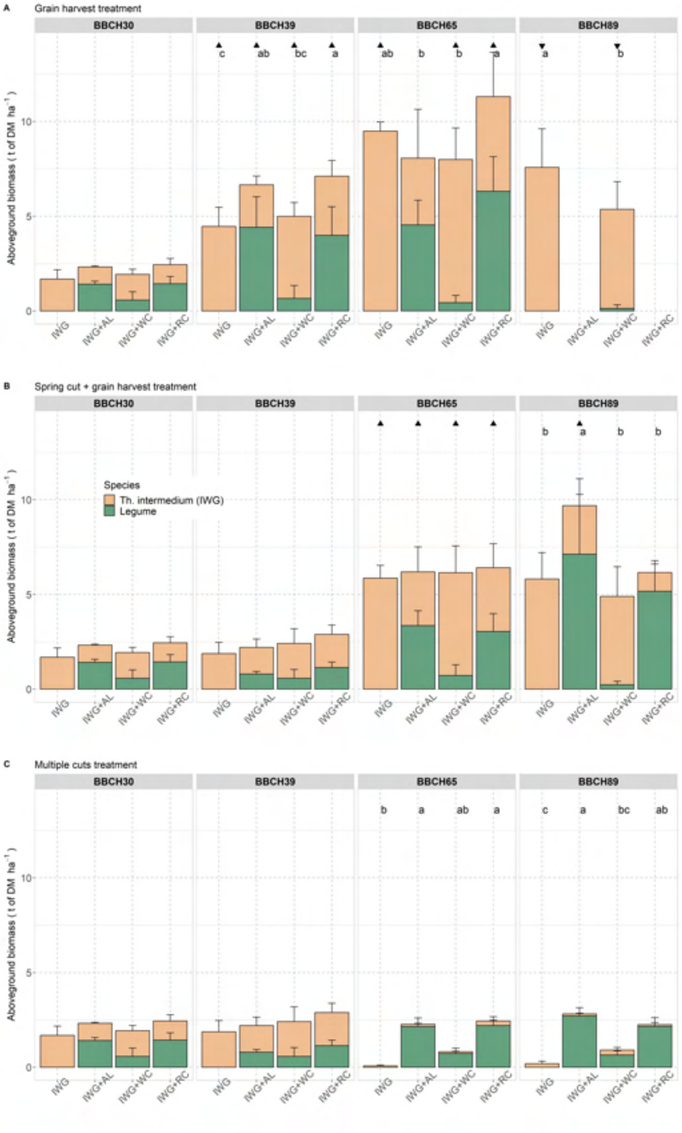

FIGURE 7.9 – Aboveground biomass production of *Th. intermedium* under various species mixture treatments and forage harvest treatments. (A) the grain harvest treatment, (B) the spring cut treatment, (C) the multiple cuts treatment. Standard errors are indicated by error bars. Letters represent the results of the post hoc analysis of the effect of the species mixture for each phenological stage (i.e., each letter is assigned to a boxplot representing the species mixture treatment). Bar plot with a symbol indicates the results of the post hoc analysis of the effect of the phenological stages for each species mixture treatment ; ▽ specifying a decreased value ; and △ specifying an increased value compared to the previous phenological stage. IWG for *Th. intermedium*, Lu for lucerne, WC for white clover and RC for red clover. For the spring cut and the multiple cuts treatments, BBCH30 corresponds to the production of the first cut (i.e., May 5, 2023). For the multiple cuts treatment BBCH39 corresponds to the production of the second cut (i.e., May 30, 2023), BBCH65 corresponds to the production of the third cut and BBCH89 corresponds to the regrowth after these three cuts.

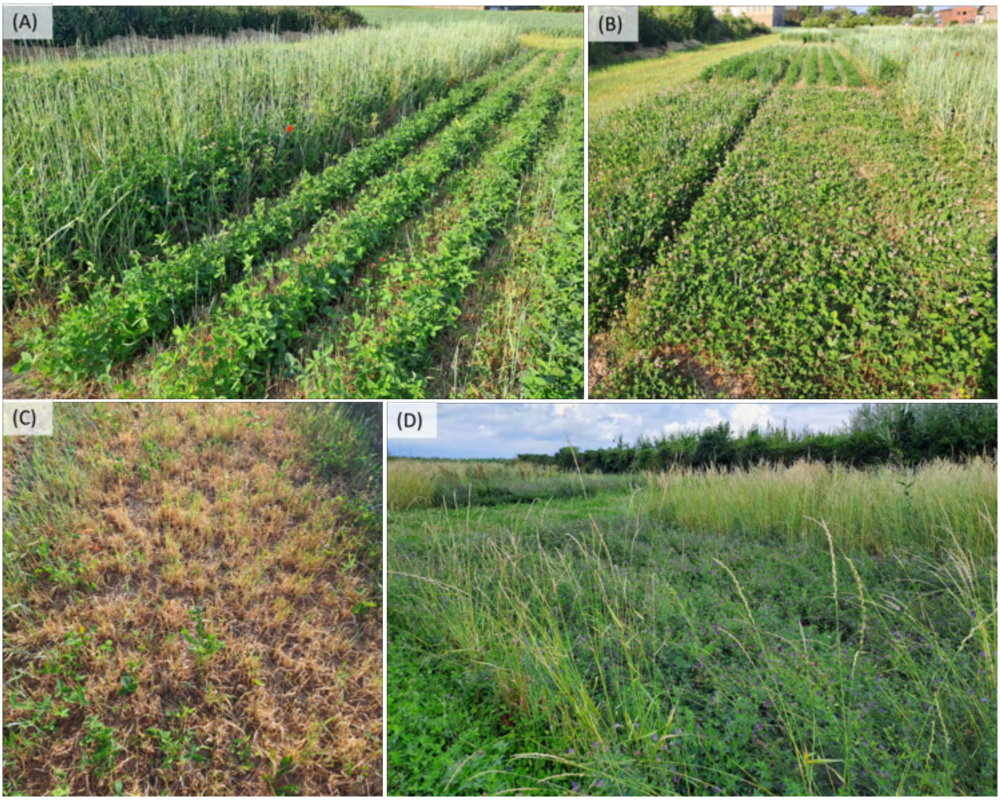

FIGURE 7.10 – Illustrations of (A) the multiple cuts treatment of *Th. intermedium* in mixture with red clover (June 13, 2023), (B) multiple cuts treatment of *Th. intermedium* in mixture with white clover (June 13, 2023), (C) the multiple cuts treatment of *Th. intermedium* in monoculture (June 13, 2023) and (D) the lodging of the spring cut treatment of *Th. intermedium* in mixture with lucerne (July 25, 2023).

In the study of Lawrence and Ashford (1966), an adverse effect of frequent defoliations on the stand of *Th. intermedium* was also observed. It was suggested that the recovery period allowed between defoliations is essential. Lawrence and Ashford (1966) observed that a 21 days recovery period yielded less than a 42 days recovery period. In our study, the recovery period between the three cuts were less than 30 days (i.e., 25 days and 29 days), probably explaining the loss of forage yields of *Th. intermedium.* As explained by Richards (1993), among the characteristics that contribute to rapid refoliation, the presence of active shoot meristematic regions remaining after defoliation is the most important.

Plant growth form influences the capacity to tolerate defoliation and to reestablish foliage and renew photosynthesis. With beneficial traits such as the protection or redundancy of apical meristems, possession of active basal intercalary meristems, higher photosynthetic rates, reduced foliage longevity, low proportion of reproductive shoots or faster rates of leaf replacement (Caldwelp et al., 1981). However, when a plant is losing its apical meristems, the activation of axillary meristems (inducing new tillers and shoots) is a slow process and is requiring sufficient resources (Hendrickson and Olson, nd). Some physiological mechanisms can be activated like accelerated photosynthetic rates after grazing or quickly move energy and nutrients throughout the plant, where grazing-tolerant grasses tended to allocate more nitrogen and carbon to shoots compared to roots (Hendrickson and Olson, nd). While the presence of active meristems after defoliation allows leaf expansion to result solely from expansion of already formed cells, rather than requiring new cell production (Richards, 1993).

Focusing on *Th. intermedium*, we observed that the proportion of reproductive tillers is relatively high, generally above 60 % (Table 6.4). As explained by Richards (1993), with this kind of grasses (i.e., caespitose grasses with synchronous tiller development) the potential to lose most active shoot meristems in one defoliation is just after internode elongation and apical meristems elevation. If a cut is performed at this time, these grasses recover very poorly while they may be quite tolerant to defoliation at other times (Richards, 1993). Lawrence and Ashford (1966) also suggested that *Th. intermedium* is in a critical phase of development during the shotblade stage and cut the crop at this stage should be avoided. In our study, the first cut was performed well before this stage, at the beginning of the stem elongation, allowing a good refoliation of the crop. Indeed, at this time, apical and axillary meristems were located near the base of the plants, inducing a better defoliation tolerance. However, the second cut was performed during apical meristems elevation inducing a very poor recovering as obviously most active shoot meristems were cut. In the study of Hendrickson et al. (2005) the effect of timing of grazing on the tiller replacement ratios (i.e., net effect of the tiller recruitment and mortality) was explored. Even if greater tiller recruitment were observed with early grazing (i.e., before late-boot stage), the number of tillers of *Th. intermedium* was decreasing during the stand life. This suggests an ability of the crop to only fill short-term forage needs where stand longevity is not a great concern. All these insights give evidence that *Th. intermedium* is not suited to intensive clipping regimes. A 40 days recovery period is at least needed between defoliations to maintain the stand and forage yields. Therefore, a first cut could be performed at the beginning of stem elongation followed by a second cut a the end of the growing season (i.e., from the flowering stage).

2.4. Conclusions

The species was not suited to close mowing during the growing season and a recovery period seemed necessary indicating that the species is more adapted to a low intensive forage use. Indeed, difficulties to initiate regrowth were observed when the apical meristems were cut. The unique forage use management seemed possible through two mowing during the growing season coupled with an autumn mowing. A spring mowing at vegetative stage can be performed characterized by low forage yield but high forage nutritive value. The second mowing can be performed at the end of the spring or summer to maximize forage yields as in any case its nutritive value is reduced after the first cut. Within this low intensive forage use, the implantation of a low competitive companion legume, such as white clover, seemed the most promising to avoid lodging risk.

8

Discussion générale et perspectives

Il convient de rappeler que les résultats discutés dans cette section ont été obtenus sous nos conditions pédoclimatiques d'Europe occidentale. Ces conditions océaniques sont relativement contrastées avec les conditions continentales Nord-Américaines d'où provient la majorité de la littérature sur l'espèce. En outre, nos différents site expérimentaux sont caractérisés par des sols profonds et fertiles (i.e., de type argilo-limoneux avec des teneurs optimales en éléments nutritifs). Enfin, leur gestion visait à maximiser le développement de la culture via, notamment, une forte régulation des compétitions telles que celles des adventices. Une exception est à mentionner dans le site BE3 et BE4 où la pression en adventices était trop forte pour pouvoir être régulée complètement.

En outre, la pérennité de la culture engendre une difficulté à dissocier l'effet des années climatiques de celui des années culturales. Par la faible variabilité des indicateurs météorologiques étudiés dans ce travail, l'effet de l'année climatique n'a pu être mesuré.

1. Ecologie fonctionnelle

1.1. Répartition des ressources entre croissance végétative et production de grains

Bien que le développement des céréales pérennes implique nécessairement la production de grains, nous avons montré que l'allocation des ressources vers les structures pérennes était essentielle pour la survie de *Th. intermedium*. L'allocation de biomasse de la plante vers les organes permanents est d'abord dirigée vers la base des tiges, puis vers le système racinaire et enfin vers les rhizomes (Figure 8.1A). Pour une culture âgée d'un à quatre ans, en termes d'azote, la base des tiges pouvait contenir de 36 à 95 kg de N ha^{-1}, les racines de 20 à 70 kg de N ha^{-1} et les rhizomes de 1 à 12 kg de N ha^{-1} (Figure 4.10). L'investissement dans les rhizomes est donc relativement réduit en ne représentant que 8 à 20 % de la biomasse souterraine (Figure 4.9). Concernant l'allocation de la plante vers les épis, celle-ci n'a représenté que 5 à 10 % de sa biomasse totale et ceux-ci contenaient de 20 à 29 kg de N ha^{-1} (Figure 8.1A et Figure 4.10). L'importance des tissus permanents peut également être soulignée par leur évolution avec le développement phénologique de la culture. Contrairement aux céréales annuelles, nous n'avons observé que peu ou pas de remobilisation des ressources vers les épis après la floraison (Figures 4.9 and 4.10) tandis que l'allocation de N a augmenté vers la base des tiges après la floraison jusqu'à l'automne. Cette faible force de puit des grains est liée aux faibles rendements en grains de la culture. En effet, au moment de la récolte des grains, la quantité de N dans la base des tiges était plus importante que celle contenue dans les épis (Figure 8.1A). Ceci suggère que la stratégie de survie de la plante dépend, en grande partie, de la base des tiges qui contient les différents méristèmes nécessaires pour le développement de nouvelles feuilles et tiges pour la saison suivante (Lemaire, 2001; Yang and Udvardi, 2018). Tout ceci confirme l'hypothèse d'un large investissement vers les structures pérennes, tout en soulignant le rôle majeur de la base des tiges pour la pérennité de l'espèce (H1, Chapitre 3).

En termes absolus, l'investissement de la plante vers les épis à la maturité des grains est resté similaire avec le vieillissement du peuplement, contrairement à sa proportion au sein de la plante. A maturité, cela représentait 26 % de la quantité totale de N de la plante lors de la première année de culture (BE4-2022) par rapport à 10 à 18 % lors des années de repousse. Ce qui est surtout expliqué par une plus faible allocation de N vers la base des tiges pendant la première année de culture (i.e., 20 % du N total à maturité, contre 29 à 39 % lors des années de repousse ; Figure 4.10). Ainsi, l'effort reproducteur semble plus important pour l'année d'établissement.

La survie de *Th. intermedium* est donc assurée via deux types de reproduction, la reproduction sexuée via la production de grains et en plus grande mesure, la reproduction asexuée (ou végétative) via la production de nouveaux ramets (ou unités génétiquement identiques à la plante mère). Pendant la saison de croissance, une certaine quantité de méristèmes, présents dans la base des tiges, sert à la production de tiges fertiles pour la reproduction sexuée. Tandis qu'une autre quantité de méristèmes doit être préservée ou nouvellement générés après la saison pour assurer la production de nouvelles tiges et feuilles (Lundgren and Des Marais, 2020). En tant que graminée cespiteuse produisant des rhizomes courts (Bajgain et al., 2020; Ogle et al., 2011), les ramets de *Th. intermedium* peuvent être des talles intravaginales (i.e., talles secondaires provenant du processus de tallage) ou des talles extravaginales (i.e., rhizomes) (Herben et al., 1994; Lafarge and Durand, 2011). Ces rhizomes ont été initiés entre le stade de la dernière feuille et de la floraison, tandis que les talles intravaginales se développent plus tôt dans la saison, de manière sylleptique (i.e., au même moment que les bourgeons en croissance ; Herben et al. (1994)). Une certaine plasticité dans la production des talles extravaginales (i.e., rhizomes) a également été observée en pouvant être plus importante sous différentes conditions environnementales (Figure 4.9). En effet, sous de faibles densités de plantes et de talles (i.e., avec une moyenne de 73 plantes m^{-2} et de

380 talles m^{-2} ; Table 4.2 et Table 4.3), une plus grande quantité de rhizomes a été formée au sein de la biomasse souterraine. Ces rhizomes ont pu être favorisés par une meilleure pénétration de la lumière, en stimulant la production de talles (tant intra- qu'extravaginales) (Langer, 1963).

La capacité de *Th. intermedium* à activer ses différents méristèmes afin de recruter des talles intra- ou extravaginales impacte directement ses performances. En effet, ces activations devraient affecter la tolérance de la plante à la défoliation ainsi que la capacité des talles à devenir reproductrices. Concernant la tolérance à la défoliation, la possession de méristèmes basaux actifs au moment de la fauche semble être un élément essentiel pour assurer une bonne repousse. Cette condition n'a été rencontrée qu'avant l'entrée en stade reproducteur (i.e., automne et début de printemps), réduisant le potentiel de production de biomasse de bonne valeur fourragère. Outre la croissance végétative, l'initiation des talles est également importante pour la production de grains de l'espèce. Pour que les talles de l'espèce soient fertiles, leur moment d'initation doit leur permettre de subir une double induction florale (i.e., besoin d'une induction primaire avec des jours courts et de faibles températures (vernalisation) et d'une induction secondaire avec des jours longs ; Duchene et al. (2021)). En outre, il est probable que ce soient les talles formées de manière précoce (i.e., début d'automne) qui produisent l'essentiel du rendement en grains comme observé pour d'autres graminées (Langer, 1980; Svečnjak et al., 2022). Ceci peut être appuyé par le fait qu'un potentiel de rendement en grains plus élevé a été constaté pour un semis précoce ayant une densité de talles en fin de saison similaire à celles des semis plus tardifs (Table 6.9). Finalement, la reproduction végétative peut détourner les ressources pour la reproduction sexuée (Yang et al., 2019). Nous avons effectivement observé une réduction du poids des grains et de la fertilité des épis avec des densités de talle élevées en début de cycle reproducteur ou avec de plus grandes proportions de talles végétatives en fin de cycle (Figure 6.6 and table 4.3). Ainsi, l'étude de la capacité de *Th. intermedium* à activer ses différents méristèmes en réponse à différents facteurs environnementaux engendrerait des avancées majeures sur la caractérisation des potentialités de l'espèce. De plus, ce trait pourrait éventuellement faire l'objet d'une sélection génétique.

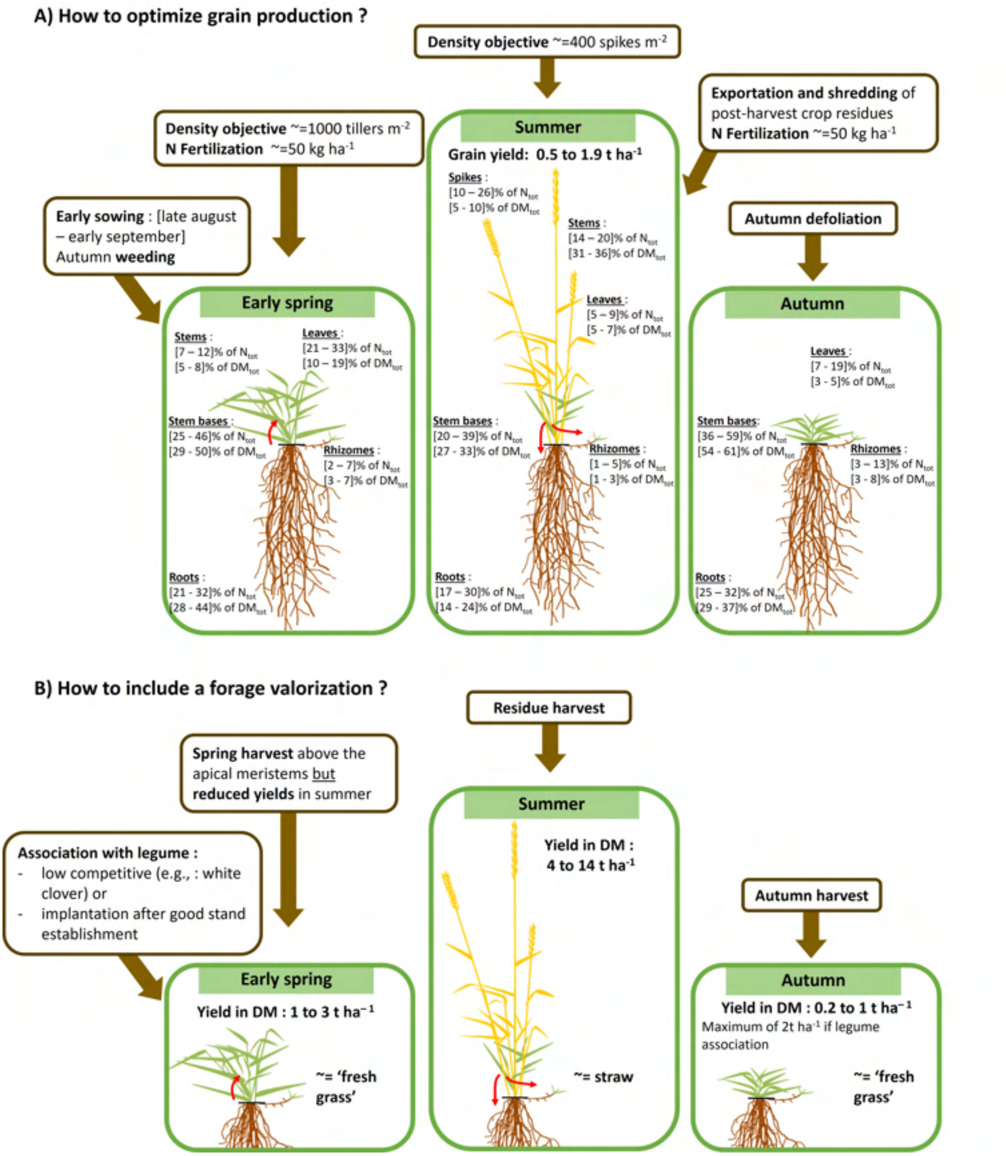

FIGURE 8.1 – Synthèses des résultats en termes d'allocations des ressources, de valorisation des productions et de gestion de la culture. N_{tot} correspond à l'azote total contenu dans la plante et DM_{tot} à la biomasse totale de la plante.

1.2. Traits fonctionnels de conservation

L'étude des traits fonctionnels peut être très utile pour identifier les synergies et les compromis entre les différents services de l'espèce. Dans ce travail, nous avons mis en évidence plusieurs traits liés à une stratégie de conservation des ressources de l'espèce. Comme expliqué par la théorie de Tilman (1982), dans des conditions de fertilité faible des sols, le taux d'acquisition des nutriments devrait être faible et les plantes devraient croitre très lentement. Les plantes qui ont la capacité de prélèvement la plus efficiente pour la ressource la plus limitante, et/ou la capacité de conserver et de stocker cette ressource au travers de mecanismes de recyclage interne efficient, devraient être les plus compétitives.

Th. intermedium, par sa forme cespiteuse, produit des ramets assez rapprochés induisant une propagation lente et radiale. Cette stratégie, appelée 'phalanx' (Doust, 1981), peut être qualifiée de conservative au contraire de la stratégie 'guerilla' qui produit des organes relativement espacés afin d'explorer et d'étendre de nouveaux ramets. Cette stratégie de croissance 'phalanx' peut induire une bonne tolérance aux conditions stressantes, une efficience d'utilisation des ressources locales (i.e., stratégie de monopolisation) et procurer un avantage compétitif sur les autres espèces présentes au sein d'un microsite (Bam et al., 2024). Cependant, nous avons observé une certaine plasticité dans les formes de croissance sous différentes conditions environnementales, ce qui a déjà été observée pour d'autres graminées (Bam et al., 2024; Hartnett, 1993). Sous une compétition intraspécifique relativement faible (i.e., densité de plantes et de talles réduite, Tables 4.2 and 4.3), *Th. intermedium* a produit une quantité plus importante de rhizomes. Ainsi, *Th. intermedium* a pu initier plus de talles 'guerilla' (i.e., rhizomes) dans cette situation, induisant une propagation plus rapide dans l'environnement (Doust, 1981).

L'efficience d'utilisation des ressources a été mise en évidence pour le N où *Th. intermedium* est capable de produire une biomasse importante avec des besoins relativement faibles en N (Figure 5.9). Notamment, le coefficient 'a' de la courbe de dilution critique de l'azote (CNDC) indique que les tissus aériens ont des pourcentages en N plus faibles que d'autres espèces (Table 5.1). Lors des récoltes estivales (exportation des tiges, feuilles et épis), l'exportation de N est faible et a varié de 60 à 80 kg N ha^{-1} (Figure 5.6, Figure 4.10). Cela représente 55 % du contenu total en N pendant la première année de croissance et 30 à 39 % pendant les années de repousse. Actuellement, la majorité du N contenu dans la plante n'est pas exporté en produits agricoles mais plutôt alloué vers les organes permanents. En particulier, la biomasse produite par le système racinaire était relativement importante, de 2 à 7 t de MS ha^{-1} (Figure 4.9), ce qui a également été observée dans d'autres études sur l'espèce (Duchene et al., 2020; Sainju et al., 2017). Ce système racinaire devrait conférer à la culture une bonne efficience de prélèvement des ressources du sol par une exploration extensive tout en limitant les pertes au travers du profil. En effet, la lixiviation des nutriments tels que le N était limitée dans l'étude de Jungers et al. (2019). Ces différents éléments se traduisent en une efficience d'utilisation de l'azote (NUE, racines incluses) importante et plus élevée que le blé annuel, comme calculé par Sprunger et al. (2018a). L'espèce semble donc capable d'assimiler de larges quantités de N, voire plus que ce qui a été appliqué par fertilisation.

Th. intermedium cultivée en chambre de culture a montré un investissement préférentiel vers la construction de ses racines (Figure 4.4H) par rapport au blé d'hiver en phase d'établissement (premiers jours de développement après semis). Au sein de la plante, la proportion plus importante de biomasse racinaire pourrait potentiellement induire une plus grande proportion de carbone respiré par rapport à la fixation photosynthétique et donc expliquer le taux de croissance relatif plus faible observé de *Th. intermedium* (Figure 4.3 ; Poorter et al. (1990)). Ce taux de croissance plus faible a induit un développement aérien et souterrain plus faible (Figure 4.4), réfutant l'hypothèse d'une importante biomasse racinaire de *Th. intermedium* en phase d'établissement (H2, Chapitre 3). Cependant, l'investissement préférentiel dans l'exploration du sol a aussi été souligné dans diverses expériences au champ. Sprunger et al. (2018a) ont par exemple trouvé que la plante allouait 23 à 50 % de sa biomasse totale vers les racines tandis que l'investissement du blé était seulement de 10 %. En outre, une densité plus élevée de tissus racinaires a été observée pour *Th. intermedium* (Figure 4.4D), ce qui pourrait refléter une stratégie de conservation des ressources, en lien avec ce qu'ont observé Duchene et al. (2020) au champ. Dans des environnements pauvres en ressources, les compétitions pour les ressources du sol prévalent (Tilman, 1985). La plus grande proportion de racines de l'espèce peut donc refléter une adaptation en facilitant la capture de ses ressources (Poorter, 1989). En outre, dans ce type de conditions, une croissance plus lente et des densités de tissus de racines, de tiges et de feuilles plus élevées sont associées à un fitness plus élevé (Kramer-Walter et al., 2016).

Cependant, les traits racinaires sont multidimensionnels avec un gradient de 'collaboration' en plus du gradient de 'conservation' (Bergmann et al., 2020; Weigelt et al., 2021). Malheureusement, cette étude ne s'est pas concentrée sur l'architecture racinaire de l'espèce, qui aurait pu différencier les multiples ordres des racines ainsi que la distribution des diamètres racinaires par exemple.

Les racines grossières (i.e., racines de transport) n'ont pas été distinguées des racines fines (i.e., racines acquisitives), conduisant à quelques difficultés pour interpréter les traits fonctionnels racinaires de 'collaboration'. En effet, dans nos conditions contrôlées en phase d'établissement, nous avons observé une longueur racinaire spécifique similaire entre les deux espèces et un diamètre racinaire plus élevé pour le blé d'hiver (Figure 4.4). Ce qui est en contradiction avec Duchene et al. (2020) qui ont observé un diamètre racinaire plus élevé et une longueur racinaire spécifique plus faible pour *Th. intermedium* en comparaison au blé et seigle annuels au champ. Ce qui suggère que l'exploration du sol moins efficiente de *Th. intermedium*, liée à une longueur racinaire spécifique plus faible, peut être compensée par une acquisition des ressources au travers d'une symbiose avec les champignons mychoriziens (AMF). Dans cette étude, une augmentation de la biomasse d'AMF sous culture de *Th. intermedium* a effectivement été observée (Duchene et al., 2020).

2. Réponse à la nutrition azotée

Grâce à l'établissement de la CNDC, nous avons pu déterminer que les besoins actuel de *Th. intermedium* en N étaient relativement faibles. La maximisation de la production de biomasse aérienne a été obtenue avec un apport de 100 à 150 kg de N ha^{-1}. Cependant, à certains stades de développement ou lors de certaines années, un apport de 50 kg N ha^{-1} a été suffisant. En effet, les coefficients apparents d'utilisation de l'azote (ANR) en fin de saison de croissance étaient relativement faibles, sous les 40 % (Table 5.11). Ainsi, par rapport au témoin sans apport de N, les traitements fertilisés n'ont absorbé, au sein de la biomasse aérienne, qu'une faible partie du N appliqué à la fin de la saison de croissance. En prenant l'exemple de l'année 2019, l'accumulation de N au sein de la biomasse aérienne a très bien répondu à la fertilisation azotée en début de saison (i.e., BBCH30) contrairement au moment de la récolte des grains (i.e., BBCH89 ; Table 5.10). Ce qui peut être lié à la chute observée de la quantité de N contenue dans la biomasse aérienne en deuxième partie de la saison de croissance (Figure 5.6).

Cette chute observée de la quantité de N aérienne est partiellement liée au partage des ressources entre les organes végétatifs et reproducteurs. En effet, la force de puit des grains est faible et la majorité de l'allocation vers les épis s'est faite du stade dernière feuille jusqu'à la floraison (Figure 4.10). Au contraire, la quantité de N au sein de la base des tiges a augmenté après la floraison ce qui pourrait être lié à une translocation de N provenant des feuilles ou des tiges ou à un nouveau prélèvement de N du sol. En outre, si on se concentre sur le N contenu dans l'ensemble de la plante (tant au niveau aérien que souterrain), celui-ci peut diminuer pendant la saison de croissance (Figure 4.10). Cette perte peut, en grande partie, être expliquée par la régression du système racinaire en fin de saison. Cependant de 22 à 28 kg N ha^{-1} ne sont pas expliqués par cette réduction de biomasse racinaire. Cette perte de N en fin de saison pourrait provenir de la rhizodeposition. Une libération de N dans le sol est possible à partir de l'exsudation des racines vivantes et/ou de l'abrasion par le sol des coiffes racinaires protégeant les zones méristématiques. Cette hypothèse a été suggérée dans l'étude de Dobbratz et al. (2023), où une augmentation du contenu en N total du sol a été observée après la récolte des grains sous culture de *Th. intermedium*. En effet, en céréales annuelles, la rhizodéposition représente en moyenne 15 % du C assimilé et une médiane de 14 % du N total assimilé par la plante (Wichern et al., 2008). Dans leur revue, Wichern et al. (2008) ont calculé que le blé pouvait laisser 68 kg N ha^{-1} dans le sol, dont 26 kg N ha^{-1} proviendrait de la rhizodeposition.

Bien que la valorisation du N en fin de saison ne soit pas totalement efficiente pour la production de grains de *Th. intermedium*, les besoins de la culture doivent tout de même être comblés pour optimiser ses multiples productions. En fin de saison de croissance, ceux-ci ont varié de 40 à 90 kg de N ha^{-1} en fonction du niveau de biomasse aérienne. Ces besoins, plus faibles qu'en milieu de saison de croissance, doivent être rencontrés par l'apport de N provenant du sol ou de la fertilisation (Figure 5.6). Dans nos conditions pédoclimatiques relativement fertiles avec des sols profonds, un apport de 100 kg de N ha^{-1} sur l'ensemble de la saison culturale semblait suffisant. Cet apport pourrait être fractionné entre la fin de l'été/ début d'automne et le début du printemps. Cette stratégie de fertilisation a été la plus efficace pour maintenir le rendement en grains constant sur quatre années (Figure 6.5). En effet, l'application de N en fin d'été pourrait améliorer l'initiation des talles reproductrices tandis que l'apport au début du printemps pourrait soutenir la production de talles reproductrices avec de larges épis et un nombre élevé de grains (Cattani and Asselin, 2017).

Ces différents éléments nous permettent de partiellement confirmer l'hypothèse d'une augmentation de la productivité de l'espèce via un apport de N pendant la saison culturale (H3, Chapitre 3). En effet, l'apport de N joue positivement sur la production végétative en début de saison, tandis que sa valorisation pour la production de grain est peu efficiente. A l'avenir, il serait intéressant d'étudier cette fertilisation azotée sur des niveaux d'apports plus fins tout comme son interaction avec les conditions pédoclimatiques. Une meilleure compréhension des facteurs pilotant les priorités d'allocation des assimilats et des nutriments vers chaque puits pendant le developpement reproducteur est nécessaire. Tout comme la contribution de chaque organe source pour augmenter la force de puits des grains de l'espèce.

3. Services de production et gestion agronomique

3.1. Production céréalière

Du fait d'une sélection génétique naissante, la production céréalière de *Th. intermedium* est actuellement faible, avec un maximum de 1,9 t ha^{-1} (Figure 8.1). Ces résultats correspondent à 10 à 20 % des rendements des céréales annuelles, également corroborés dans d'autres études sur l'espèce (Culman et al., 2013; Larkin et al., 2014; Newell and Hayes, 2017). Ces faibles rendements en grains sont d'abord expliqués par un faible rendement par épi (fonction du poids de mille grains (PMG) et de la fertilité d'épi). Les grains de *Th. intermedium* sont relativement petits, avec un PMG maximal de 9.6 g tandis que la fertilité d'épi a varié de 17 à 35 grains $épi^{-1}$ (Figure 6.5). Comme observé par Altendorf et al. (2021), la fertilité des fleurons n'excède pas 49 % et l'utilisation de ces unités de reproduction n'est pas optimale représentant une des principales limitations pour la production de grains (Larson et al., 2019). Cependant, ce trait est complexe et peut être influencé par de multiples facteurs comme la disponibilité du pollen, l'auto-incompatibilité, l'égrenage ou l'avortement des grains (Altendorf et al., 2021).

Bien que faible par rapport aux céréales annuelles, cette valeur de rendements en grains fait partie des valeurs les plus élevées connues pour l'espèce. Il convient donc de rappeler que les conditions pédoclimatiques de notre étude sont bien différentes des conditions continentales Nord-Américaines d'où provient la majorité de la littérature sur l'espèce. Nos conditions océaniques d'Europe occidentale ont été relativement propices au développement de la culture. Notamment via des indicateurs météorologiques plutôt favorables (Table 6.2). En outre, le site expérimental est caractérisé par un sol profond et fertile dont les teneurs en éléments nutritifs étaient optimales sur les quatre années de culture (i.e., avec en moyenne 0,16 mg g^{-1} de phosphore, 0,22 mg g^{-1} de potassium et 13 g kg $^{-1}$ de carbone organique).

La chute du rendement avec le vieillissement du peuplement représente un blocage important en termes de potentiel de production céréalière de l'espèce. Plusieurs hypothèses avaient précédemment été émises par certains auteurs pour expliquer la baisse de rendement lors des années de repousse de la culture. Notamment, une concurrence accrue pour la lumière ou les nutriments avec une densité de tiges plus élevée (Jungers et al., 2017; Tautges et al., 2018) ou encore des changements dans l'allocation des ressources en faveur des organes pérennes (Vico et al., 2016). Comme précédemment discuté, en première année de culture, la proportion de ressources allouées vers la reproduction sexuée était effectivement plus importante (Figure 4.10). Lors de la première année, le PMG et le contenu en N des grains étaient également les plus élevés (Figure 6.5). En outre, nous avons observé que lorsque l'allocation des ressources vers la base des tiges augmentait (lors de certaines années de repousse), les rendements en grains diminuaient (Figure 4.10 and table 4.3). Cela peut être expliqué par une plus faible mortalité de talles et une plus grande proportion de talles végétatives, ce qui peut détourner les ressources des épis en croissance (Yang et al., 2019). Par ailleurs, la densité des talles et la biomasse aérienne en début du cycle reproducteur ont également été observées comme détrimentaires pour le rendement par épi en impactant négativement le PMG et la fertilité des épis (Figure 6.6). Lors de l'année d'établissement, un investissement dans la production de talles, tant intra- qu'extravaginales a été observé. Cette augmentation de la densité de talles jusqu'à la récolte (Figure 6.3 and table 4.3) peut mener à une biomasse maximale en deuxième année de culture (Figure 6.2), comme également observé dans d'autres études (Duchene et al., 2023). Ces excès de biomasse doivent donc être contrôlés en évitant des densités de talles trop élevées, ce qui a également été constaté dans l'étude de Hunter et al. (2020a).

Un résultat majeur de nos recherches est la possibilité de maintenir ces rendements en grains constants avec le vieillissement du peuplement, ce qui n'a jamais été observé dans d'autres études sur l'espèce. Une valeur moyenne de 1 t ha^{-1} de grains a été observée sur quatre années de culture (Figure 6.5). En effet, ce rendement est expliqué par une multitude de facteurs et peut être piloté via la gestion agronomique de la culture. Nous avons démontré que la densité de grains était le contributeur majeur du rendement en grains, comme pour les céréales annuelles (Hay and Porter, 2006). Ces différents résultats confirment l'hypothèse d'un investissement réduit dans la reproduction sexuée tout en partageant des mécanismes similaires aux céréales annuelles (H4, Chapitre 3). Ainsi, pour maintenir cette densité de grains, l'induction florale des talles pour obtenir une densité optimale d'épis est essentielle. Toutefois, des mécanismes de compensation ont été observés où une densité trop élevée d'épis peut nuire à leur fertilité (Figure 6.6). Une bonne densité devrait donc tourner autour des 400 épis m^{-2}, sans dépasser les 700 épis m^{-2}, comme également conseillé dans l'étude de Hunter et al. (2020a).

En termes d'implantation, une densité de semis avoisinant les 20 kg ha^{-1} avec des interlignes relativement étroits (i.e., autour de 20 cm) et une date de semis précoce (i.e., fin aout – début septembre) ont été bénéfiques pour assurer un bon établissement de la culture en favorisant sa compétitivité et en maximisant le nombre potentiel de talles reproductrices (Tables 4.2 and 4.3). En effet, des semis relativement denses peuvent potentiellement contraindre la production de rhizomes. Ce qui pourrait limiter la production de talles qui vont se développer plus tard dans la saison et rester végétatives au détriment du rendement en grains (Figure 4.9 et Table 4.3). En outre, nous avons observé qu'un semis précoce maximisait la fertilité des talles et le rendement en grains final (Table 6.9).

Lors des années de repousse, il est crucial de maintenir le potentiel reproducteur des talles en évitant les excès de biomasse végétative et en favorisant l'induction florale des talles. Ainsi, la fertilisation azotée peut être fractionnée entre l'automne et le début du printemps (i.e., BBCH29) en apportant 50 kg de N ha^{-1} à chaque fraction, comme précédemment discuté. Cependant, cette généralité devrait être adaptée en fonction des conditions locales et du développement observé de la culture. Par exemple, si la production de biomasse de *Th. intermedium* est assez importante en début de saison (BBCH30), et que le nombre de talles est déjà suffisant (i.e., dans nos conditions avec une densité de talles autour de 1300 talles m^{-2}), la fraction azotée de début de saison devrait être réduite. En outre, en plus de détourner les ressources du grain, la production excessive de biomasse végétative peut engendrer de la verse en fin de saison (Jungers et al., 2017).

De précédentes études ont mis en évidence un déclin du rendement avec le vieillissement du peuplement lié à une diminution de la densité d'épis (Jungers et al., 2017 ; Law et al., 2020). Cette diminution pourrait être expliquée par un changement de la qualité de la lumière perçue par les méristèmes axilliaires situés à la base des plantes. Ce qui diminuerait l'initiation reproductive des talles ou déclencherait le syndrome dévitement de la lumière (Jungers et al., 2017; Pinto et al., 2021; Rouet et al., 2021). Ainsi, la bonne gestion des résidus post-récolte (e.g., exportation des résidus et broyage au ras du sol) peut aider à favoriser la pénétration de lumière à la base des tiges. Celle-ci peut également être favorisée par la fauche à l'automne, qui a également eu pour effet de réduire la biomasse aérienne au printemps suivant et la densité de talles en fin de saison ce qui a augmenté le PMG (Table 6.3). Ainsi, la fauche d'automne peut limiter la quantité excessive de biomasse à la sortie de l'hiver sans nuire au potentiel reproducteur (voire même en augmentant le rendement par épi) par un prélèvement suffisamment précoce de la surface photosynthétique (Figure 8.1 ; Culman et al. (2023); Pugliese et al. (2019)). Ce qui réfute l'hypothèse d'une réduction de l'allocation reproductive par une exploitation fourragère d'automne (H7, Chapitre 3).

Finallement, comme mentionné par Altendorf et al. (2021), la sélection pour le rendement en grains de *Th. intermedium* devrait se concentrer sur la réduction du tallage excessif pour permettre une meilleure allocation des ressources vers les épis. En outre, l'identification du moment optimal de perception de la lumière au niveau des méristèmes pour l'induction des talles reproductrices devrait être identifié. En effet, l'approfondissement des recherches sur la dynamique de tallage de l'espèce pour trouver les conditions favorables au renforcement de la croissance des talles reproductrices semble essentielle.

3.2. Production fourragère

Concernant sa production fourragère, celle-ci est relativement limitée. Dans une gestion à double usage grain-fourrage, il est possible de valoriser de la biomasse avec une bonne valeur fourragère au début du printemps et à l'automne. Cette biomasse est proche d'une herbe fraîche qui peut être utilisée pour l'alimentation de vaches laitières en lactation (Figure 8.1 and table 7.5). Cependant, la production de biomasse cumulée de ces deux fauches n'a pas dépassé les 2 t de MS ha^{-1} (Figure 7.5). La paille produite lors de la récolte des grains peut également être récoltée avec une valeur fourragère faible mais des quantités de biomasse intéressantes, qui ont variés de 5 à 15 t de MS ha^{-1} (Figures 6.2 and 8.1). Dans un mode de gestion uniquement fourrager, seulement deux coupes avec une biomasse avoisinant à chaque coupe 1,8 t de MS ha^{-1} ont pu être réalisées sur une saison de croissance. La repousse à la suite de ces deux coupes était quasiment inexistante (Figure 7.9), ce qui a réduit le potentiel de rendement (en biomasse aérienne) cumulé sur la saison de moitié par rapport à des modalités conduites pour la production de grains et de fourrage. En outre, seule la première coupe avait une bonne valeur fourragère (i.e., proche d'une herbe fraîche) tandis que la deuxième coupe a été de qualité moyenne proche d'un foin de pauvre qualité fourragère (Tables 7.5 and 7.6). En effet, après la première coupe, les tiges reproductrices ont poursuivi leur élongation donnant un fourrage très fibreux. La deuxième coupe a, elle, eu pour effet de couper le méristème apical des tiges induisant une repousse très faible.

L’aptitude des graminées fourragères à rétablir leur surface photosynthétique après les coupes est dépendante des réserves présentes dans la base des tiges et de l’activation des bourgeons axillaires pour la production de nouvelles tiges et feuilles (Caldwelp et al., 1981; Hay and Porter, 2006). Ainsi, la forme de croissance de la plante va influencer sa capacité à tolérer la défoliation. *Th. intermedium* est une graminée cespiteuse avec un développement des talles relativement synchrone. En fauchant la culture lors de l’élongation de l’épi dans la tige, la majorité des méristèmes aériens actifs sont perdus, induisant une très mauvaise repousse (Richards, 1993). Les études de Lawrence and Ashford (1966) ainsi que Hendrickson et al. (2005) ont aussi suggéré que les coupes ne devaient pas être réalisées autour de la période de la dernière feuille. Cependant, nous avons observé que le N contenu dans la base des tiges avait tendance à réaugmenter après la floraison (Figure 4.10) tandis que la plante est capable de repousser après la récolte des grains. Ce qui suggère que la deuxième coupe ne devrait pas se faire avant la floraison.

Ainsi, la culture n’est pas adaptée à un régime de coupes multiples au cours de la saison de croissance. Une seule coupe au début du printemps, avec une bonne valeur fourragère mais un rendement faible, peut être réalisée. Un temps suffisant d’accumulation de réserves avant la deuxième coupe doit ensuite être respecté. Cette deuxième coupe devra donc être réalisée à un stade reproducteur avancé, induisant une faible valeur fourragère (i.e., proches de pailles de graminées fourragères ou de céréales ; Table 7.5) mais un rendement relativement élevé. Finalement, une coupe d’automne pourra être réalisée avec une qualité fourragère intéressante mais un rendement généralement très faible (Figure 7.5). L’hypothèse d’une exploitation uniquement fourragère (H6, Chapitre 3) est donc réfutée puisque cette gestion ne permet pas d’intensifier la production fourragère de l’espèce par rapport à une gestion double usage. En outre, l’hypothèse d’un intérêt pour une exploitation fourragère additionnelle à l’exploitation du grain peu partiellement être validée (H5, Chapitre 3). En effet, par une production majoritaire de fourrage de faible valeur nutritive, sa valorisation devrait être extensive.

4. Potentiels d’intégration dans les agroécosystèmes

4.1. Phase d’implantation et gestion de la compétition

Afin d’observer la physiologie de l’espèce en phase d’établissement, nous avons comparé son développement à celui d’une céréale annuelle largement cultivée telle que le blé d'hiver. Cultivée en chambre de culture, la dynamique de croissance de la céréale pérenne lors du développement juvénile était plus tardive (Figure 4.3), en lien avec une stratégie de conservation des ressources et une colonisation lente de son environnement. Cette phase lente d'établissement peut amener certaines difficultés au champ, telle qu'une certaine pression en adventices liée à une compétitivité faible de la culture via une production végétative réduite de biomasse comme observé à la Figure 4.9 en 2022. Cela avait déjà été mis en évidence par Duchene et al. (2023). Dans son étude, les adventices ont été très concurrentielles dès les premiers mois de développement de la culture. La biomasse d'adventices était relativement élevée au printemps, autour de 1 à 2 t de MS ha^{-1}, ce qui induit une nécessité de désherbage dès l'automne pour limiter l'effet dépressif des adventices sur la culture. Cette faible compétitivité de la part de *Th. intermedium* a également été observée au sein de différentes associations à des légumineuses, via une réduction de sa production de biomasse aérienne et de son rendement en grains. Lorsque la céréale pérenne était associée à de la luzerne ou du trèfle violet, ces pertes de production étaient de 1 à 5 t de MS ha^{-1} et de 37 à 100 % pour le rendement en grains (Figures 7.5 and 7.8). Ce qui a également été observé dans d'autres études (Tautges et al., 2018; Pinto et al., 2022).

Un bon développement de la culture dès le semis doit être favorisé (i.e., semis soigné et précoce) pour éviter une concurrence immédiate sur la culture de *Th. intermedium*. En effet, cette concurrence de plantes volontairement présentes ou non dans la parcelle peut se poursuivre lors des années de repousse de la culture. Contrairement aux prairies temporaires, le levier des fauches régulières pour réguler la pression des adventices, surtout annuelles, ne peut être utilisé avec la culture de céréale pérenne. Duchene et al. (2023) ont donc observé que les populations d'adventices se sont spécialisées avec le vieillissement de culture. Ces populations d'adventices se sont adaptées pour assurer leur survie dans la culture de céréale pérennes (i.e., graminées, espèces pérennes et plus précoces que *Th. intermedium*), rendant leur élimination encore plus complexe. Comme observé dans les études de Law et al. (2022) et Duchene et al. (2023) où la production de biomasse par les adventices au printemps a été constante sur les différentes années de culture.

Ainsi, différents leviers doivent être actionnés pour limiter la concurrence de ces adventices tout en étant adaptés à chaque historique et contexte spécifique. En agriculture conventionnelle, le désherbage chimique sur l'ensemble de la parcelle ou de manière localisée pour certaines adventices problématiques (i.e., tels que des vivaces ou des graminées) pourrait être utilisé. Cette technique a été la plus efficace dans nos essais. Cependant, la résistance de l'espèce aux multiples molécules herbicides utilisées en prairie ou en céréales annuelles reste encore à déterminer. Une autre solution de désherbage est l'utilisation d'outils mécaniques. Le déserbage entre les rangs est possible en utilisant une bineuse si ceux-ci sont adaptés (i.e., minimum 20 cm d'écartement). L'utilisation d'outil en plein, comme la herse étrille par exemple, semble peu adaptée lorsque la culture est en phase d'établissement. En effet, une certaine sensibilité de *Th. intermedium* au recouvrement a été observée au moment où le passage de l'outil était possible (i.e., stade deux ou trois feuilles). Finalement, l'association à des légumineuses (ou d'autres espèces) pourrait également être envisagée pour réduire le développement de nouvelles adventices en limitant l'accès aux ressources. Cependant, ces légumineuses devront être contrôlées pour limiter les effets dépressifs sur la culture, en développant par exemple des outils pour la fauche au sein de l'inter-rang. La conception de nouveaux systèmes de culture semble donc fondamentale.

4.2. Particularités liées à la multifonctionnalité de la culture

En tant que culture multifonctionnelle, *Th. intermedium* offre divers services écosystémiques au-delà de la production de grains, tels que la production de fourrage, la protection des ressources du sol, la régénération de la santé des sols, la conservation de la biodiversité ou l'amélioration de la résilience de l'agroécosystème (Duchene et al., 2019; Ryan et al., 2018). Cela traduit un certain compromis entre l'intensification des services de production et des services de soutien à l'environnement. La prise en compte de cette multifonctionnalité semble nécessaire pour l'introduction de ces céréales pérennes dans les systèmes de culture européens et implique actuellement une valorisation extensive des services de productions.

4.2.1. Stratégie de valorisations à double usage

La valorisation uniquement fourragère de la culture, comme précédemment discutée, n'a pas permis d'augmenter le potentiel fourrager de l'espèce par rapport à une gestion double-usage grain et fourrage. En effet, la plante n'est pas adaptée à des régimes de coupes fréquentes (Heinrichs and Clark, 1961; Campbell, 1961). Ainsi, pour permettre la survie de *Th. intermedium*, la majorité de la biomasse fourragère doit être récoltée en fin de cycle reproducteur (Figure 7.9), ce qui induit une faible qualité fourragère. Les stratégies les plus pertinentes de gestion de la culture seraient donc à double usage. Pour ce faire, des fauches additionnelles à la récolte des grains peuvent se faire en début de printemps et à l'automne et sont caractérisées par de bonnes qualités fourragères. Cependant, l'hypothèse d'une réduction de l'allocation reproductive a été confirmée avec la fauche de printemps (contrairement à la fauche d'automne, H7, Chapitre 3). En effet, elle a eu pour effet de diminuer les rendements en grains, comme observé dans le chapitre 6 (section 1.3.3). Cela pourrait être expliqué par le prélèvement de la surface foliaire essentielle pour la production de grain couplé à une accumulation de réserves limitée après la fauche (Culman et al., 2023). En outre, dans nos conditions, la repousse à l'automne est généralement trop faible pour justifier une fauche. Une solution pour ce manque de productivité à l'automne serait l'implantation d'une légumineuse, ce qui a permis une augmentation significative de la production de biomasse à cette période (Figure 7.5). Cette légumineuse devrait être peu compétitive vis-à-vis de *Th. intermedium* en produisant une biomasse modérée, comme c'est le cas pour le trèfle blanc, ou devrait être implantée après la première année pour maximiser l'établissement de la céréale pérenne.

Ainsi, la meilleure stratégie de gestion à double-usage de la culture serait une valorisation de biomasse au moment de la récolte des grains et une fauche à l'automne, ce qui n'engendrerait pas de risques de réduction des rendements en grains. Une quantité limitée de fourrage à haute valeur fourragère est donc produite pendant une saison de croissance. La valorisation du fourrage par les herbivores devrait se faire dans le cadre d'élevages extensifs avec des objectifs de production modérés. Comme le mentionnent Duchene et al. (2021), *Th. intermedium*, en tant qu'espèce à croissance lente, pourrait être mieux adaptée aux conditions pédoclimatiques difficiles (e.g., les champs à haute altitude ou à faible disponibilité de ressources) grâce à sa capacité à produire des niveaux élevés de biomasse avec de faibles besoins en ressources telles que l'azote (Fagnant et al., 2023).

Par la faible quantité de grains produits, ceux-ci devraient être valorisés dans des marchés de niches pour l'alimentation humaine afin de maximiser la valeur ajoutée. En Amérique du Nord, ces grains sont notamment commercialisés par Perennial Pantry où les services écosystémiques sont mis en avant comme le renforcement de la santé des sols ou la lutte contre le réchauffement climatique ainsi que d'autres arguments tels que le goût délicieux des grains (Perennial Pantry, nd). Bien qu'il n'y ait pas encore de marché européen, les investissements américains dans la transformation agroalimentaire d'entreprises comme General Mills ou Patagonia Provisions sont prometteurs (Duchene et al., 2019). Finalement, une certaine valeur pourrait également être dégagée des services environnementaux fournis par l'espèce. Comme mentionné par Duchene et al. (2019), les agriculteurs européens pourraient recevoir des paiements provenant de la PAC ou accéder à de nouveaux marchés tels que les crédits carbone (Pinto et al., 2022).

4.2.2. Objectifs de sélection et compromis

Comme souligné par DeHaan et al. (2023), un long travail de développement de céréales pérennes est encore nécessaire via un investissement soutenu avant de pouvoir complètement intégrer ce type de culture au sein des agroécosystèmes. La sélection génétique, la création de nouveaux systèmes de culture productifs et l'étude des potentiels risques et avantages inhérents sont essentiels.

Depuis 2003, le Land Institute (Kansas, Etats-Unis) sélectionne des populations améliorées de *Th. intermedium*. Plus récemment, d'autres programmes de sélection ont vu le jour dans des universités nord-américaine et suédoise. Les objectifs de sélection sont notamment tournés vers le pourcentage de graines nues, le rendement par épi (i.e., fertilité des fleurons, poids du grains), la résistance à l'égrenage et à la verse, la taille réduite des plantes, la vigueur de développement au printemps, la synchronisation de floraison ou encore une maturité précoce (Bajgain et al., 2022). D'importants progrès ont été réalisés, comme estimé par Bajgain et al. (2022), l'augmentation du rendement en grains par cycle de sélection est de 58 kg ha^{-1}. Ces programmes de sélection bénéficient actuellement de nouvelles techniques moléculaires. L'accès au génotypage par séquençage et à l'ébauche du génôme a permis aux sélectionneurs de développer la sélection génomique. Le temps nécessaire pour atteindre des rendements similaires au blé annuel dépendra des futurs progrès génétiques et des améliorations agronomiques pour la gestion de cette nouvelle culture. Il a été estimé que 69 % de l'augmentation du rendement devrait provenir de cette sélection et qu'il faudrait approximativement 33 ans pour atteindre les moyennes de rendement en grains du blé annuel au Kansas, Etats-Unis. Une cinquantaine de cycles de sélection devraient être nécessaires pour atteindre un poids de grain de 30 mg (Bajgain et al., 2022).

En tant qu'espèce auto-incompatible, la sélection pour l'uniformisation de la floraison devrait améliorer son potentiel de production de grain. L'objectif serait d'obtenir une floraison uniforme à des moments optimaux pendant la journée, pour éviter l'indisponibilité du pollen, et pendant la saison de croissance, pour éviter les stress thermiques et hydriques (Cattani and Asselin, 2018). Comme historiquement réalisé pour les céréales annuelles, un autre objectif de sélection pourrait être la hauteur de plantes qui est généralement supérieure à 1,5 m en fin de cycle (Table 6.4). Cette hauteur élevée engendre des difficultés lors de la récolte où beaucoup de matière doit être battue pour récupérer les grains et peut faciliter les phénomènes de verse. En outre, une plus petite taille pourrait potentiellement induire une meilleure répartition des ressources au sein de la plante et faciliter l'allocation vers les grains (Hay and Porter, 2006). Outre la sélection, une gestion agronomique adaptée peut également contribuer à l'amélioration des rendements. Concernant la hauteur des plantes par exemple, l'utilisation de régulateurs de croissance (Frahm et al., 2018), la réduction de densité de plantes ou une fertilisation azotée modérée pourrait aider à limiter les risques de verse.

Le processus de sélection de l'espèce pour l'augmentation de la force de puit des grains ne devrait pas compromettre ses bénéfices environnementaux. Cela a été étudié par Vico et al. (2016) qui ont mis en évidence une invariance de l'allocation de biomasse des cultures pérennes en cours de sélection génétique soutenant une allocation importante vers les organes souterrains par rapport aux espèces sauvages, ce qui devrait garantir leur multifonctionnalité. Au contraire, Pastor-Pastor et al. (2019) ont comparé deux genres de *Physaria* comme modèle pour la domestication de plantes pérennes. Un premier genre était sélectionné pour sa stratégie acquisitive et à haut rendement tandis que le deuxième était son homologue sauvage. Les plantes à haut-rendements ont maitenu des taux d'acquisition en N élevés mais les réserves dans les racines étaient plus faibles que les accessions sauvages. Cela pourrait donc affecter la longévité et la stabilité du rendement des plantes au cours de la sélection. Concernant l'allocation de N, Gonzalez-Paleo et al. (2023) ont comparé des lignées sauvages et des lignées en cours de sélection pour le rendement en grains de *Silphium integrifolium* Michx. et ont identifié des changements dans l'efficience d'utilisation du N. Les individus où le rendement en grains a été amélioré ont une plus grande allocation de N vers les feuilles et les grains avec un plus faible stockage de N dans la base des tiges. Cela peut induire un besoin plus important d'apport de N externe par rapport aux lignées sauvages. Actuellement, les besoins en intrants azotés sont relativement réduits avec des exportations faibles de N au moment de la récolte des grains (Figures 4.10 and 5.9), qui pourraient donc augmenter avec la sélection pour des rendements en grains plus élevés.

Au sein de *Th. intermedium* un certain compromis existe entre la production fourragère et céréalière. La densité de talles, bien que pouvant représenter un frein pour la production de grains, est pourtant nécessaire pour le développement végétatif de la culture. Cette densité de talles devrait permettre de produire une quantité de biomasse aérienne intéressante pour la valorisation fourragère. Tandis qu'une plus grande proportion de talles végétatives permettrait qu'un plus grand nombre de méristèmes actifs soient situés dans la base des tiges. Cette proportion potentiellement plus élevée de méristèmes dans la base des tiges faciliterait la repousse de la culture au printemps et permettrait d'intensifier l'exportation de biomasse de bonne valeur fourragère. A l'opposé, comme suggéré par Altendorf et al. (2021), le processus de sélection pour l'augmentation du rendement en grains de *Th. intermedium* devrait écarter les plantes qui tallent de manière agressive. L'objectif serait de favoriser les plantes avec une proportion élevée de talles reproductrices et qui maximisent le rendement par épi avec des densités de talles plus faibles. Le potentiel de production végétative pour l'exploitation fourragère serait donc potentiellement réduit. Il semble donc difficile de sélectionner l'espèce à la fois pour la production de fourrage et la production de grains. En fonction des objectifs de l'agriculteur, la création de cultivars, à vocation principalement céréalière ou à vocation céréalière et fourragère extensive pourrait potentiellement être envisagée.

Outre la domestication de *Th. intermedium*, certaines avancées dans les blés hybrides pérennes ont récémment été mises en avant (The Land Institute, 2023) où une première variété de blé pérenne a survecu deux années au champ. Les emplacements chromosomiques exacts qui contrôlent la pérennité et l'annualité dans certaines lignées de blé pérenne ont été identifiés. Avec plus de travail, le programme de recherche sur le blé pérenne pourra déterminer si la modification du gène conférant le trait annuel pourrait permettre à la culture de repousser pendant plusieurs années pour, au fil du temps, convertir des blés annuels en pérennes. Finalement, d'autres candidats auraient un potentiel pour devenir des céréales pérennes à haut rendement. Comme testé par Westerbergh et al. (2018), *Hordeum bulbosum* possède une compatibilité croisée avec l'orge annuelle. Ainsi, elle représente une ressource génétique intéressante pour le développement d'orge pérenne en utilisant la domestication ou l'hybridation.

9

Conclusions

L'intérêt de l'introduction de céréales pérennes dans nos agroécosystèmes réside dans leur multifonctionnalité en procurant des services de production via le grain et le fourrage ainsi que des bénéfices environnementaux. Son investissement important dans le système racinaire et les organes pérennes ont permis à *Th. intermedium* de réduire certaines externalités comme l'azote. En outre, l'espèce exporte une faible proportion d'azote en fin de saison. Quelques traits de conservation des ressources ont également été observé via une densité plus élevée de tissus racinaires et un investissement préférentiel vers ses racines en phase d'établissement. Ce qui peut expliquer un taux de croissance relativement plus faible par rapport à une céréale annuelle comme le blé d'hiver. Finalement sa forme cespiteuse, avec des ramets rapprochés, induit une propagation lente et radiale de la plante. Ce qui pourrait lui conférer une bonne tolérance aux conditions stressantes ou une efficience d'utilisation des ressources locales (i.e., stratégie de monopolisation).

Cette multifonctionnalité engendre également divers compromis. Un investissement important dans les organes permanents, lui assurant une reproduction végétative solide, peut limiter les autres services de production de la culture tels que la production de grains. En effet, les excès de biomasse et de densité de talles en début de saison ont impacté négativement le rendement par épi. Bien que cette production de grains soit actuellement faible, un résultat majeur est qu'elle peut rester constante avec le vieillissement du peuplement grâce à une fertilité de talle et d'épi constante. Pour maximiser la fertilité des talles, un semis relativement dense à la fin de l'été, un broyage des résidus de récolte au niveau du sol et une fertilisation de 100 kg de N ha^{-1} fractionnée à l'automne et en début de printemps ont été intéressants. Finalement, une défoliation à l'automne peut également prévenir l'obstruction de la lumière au niveau de la base des plantes et éviter la surproduction de biomasse la saison suivante.

La gestion à simple usage fourragère n'a pas permis d'intensifier les performances de l'espèce. Lors de la phase reproductrive, une difficulté à initier la repousse lorsque les méristèmes apicaux sont coupés a été observée. La gestion fourragère semble donc uniquement pertinente dans un contexte à double valorisation grain-fourrage. Les fauches de printemps et d'automne ont été caractérisées par des rendements faibles mais avec une bonne qualité fourragère à l'opposé du fourrage produit au moment de la récolte des grains. Dans ce contexte, la majorité de la biomasse produite aura une qualité fourragère faible. En outre, la fauche de printemps est à éviter car elle a réduit le rendement en grains tandis que la repousse à l'automne a généralement été trop faible pour justifier une fauche. Tout ceci suggère une valorisation de la biomasse au sein d’élevages extensifs avec des objectifs de production modérés.

La conception de nouveaux systèmes de culture est donc essentielle afin d’optimiser les productions de la céréale pérenne. Celle-ci pourrait par exemple être associée à une légumineuse peu aggressive pour augmenter les quantités de biomasse à l’automne. En outre, la phase d’établissement de l’espèce est très lente et nécessite de trouver de nouvelles solutions pour limiter la concurrence des plantes désirées ou non au sein de la parcelle. Enfin, la sélection génétique devrait aider à la création de nouveaux cultivars adaptés aux différents contextes de production des agriculteurs intéressés par l’implantation de ce type de culture. Certains cultivars pourraient par exemple être dédiés principalement à la production de grains et de services environnementaux. Tandis que d’autre cultivars pourraient être adaptés à la double production extensive de grains et de fourrage. La sélection pour le rendement en grains devrait se tourner vers l’augmentation du rendement par épi et la limitation du nombre de talles (tant par tallage que par production de rhizomes), sans compromettre les densités d’épis, la vigueur de repousse et les performances environnementales.

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