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Identification and genotypic variability of plant traits early determining nitrogen use efficiency (NUE) in winter oilseed rape under low-N inputs

Victor Vazquez-Carrasquer

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Identification and genotypic
variability of plant traits early
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(NUE) in winter oilseed rape
under low-N inputs

Thèse de doctorat de l'université Paris-Saclay

École doctorale n° 581 : agriculture, alimentation, biologie,
environnement et santé (ABIES)

Spécialité de doctorat: Sciences agronomiques

Unité de recherche : Université Paris-Saclay, INRAE, AgroParisTech, UMR ECOSYS,
78850, Thiverval-Grignon, France.

Référent : AgroParisTech

Thèse présentée et soutenue à Paris-Saclay, le 26 janvier 2021, par

Victor VAZQUEZ-CARRASQUER

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Invité

Als meus avis,

Joan Carrasquer Cubota i Pilar Estrada Aymerich

R.I.P

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I conducted this Ph.D. in INRA research center at the ECOSYS research unit (Functional ecology and ecotoxicology of agroecosystems) directed by Dr Enrique Barriuso. I worked in a team entitled «Eco&Phy» leader by Dr Benjamin Loubet. My PhD was directed by Dr Michaël Chelle (Research Director) under the co-supervision of Dr Celine Richard-Molard (Senior Research Fellow) and Anne Laperche (Senior lecturer) and the scientific support of Dr Christine Bissuel-Belaygue (Senior lecturer).

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RÉSUMÉ DE THÈSE DE DOCTORAT EN LANGUE FRANÇAISE

TITRE DE LA THESE : Identification et variation génotypique des traits déterminant précocement l'efficacité d'utilisation de l'azote (NUE) du colza d'hiver dans des conditions à faible disponibilité en azote.

ORGANISATION DU MANUSCRIT

Le manuscrit de thèse est organisé en une introduction générale, un premier chapitre présentant les objectifs de la thèse et les stratégies employées, et un deuxième chapitre présentant une synthèse bibliographique. Ensuite figurent deux chapitres de résultats, chacun basé sur un article en anglais soumis dans une revue scientifique (Chapitre III) ou destiné à une soumission future (Chapitre IV). Le manuscrit se termine par un chapitre de discussion générale et de perspectives. Le manuscrit de thèse a été rédigé entièrement en anglais.

CHAPITRE I. INTRODUCTION ET PRESENTATION DE LA THESE

La fertilisation azotée (N) a permis une augmentation des rendements des cultures depuis plusieurs décennies. Cependant, l'application excessive d'engrais azotés constitue un problème environnemental majeur. Les pertes d'azote par le lessivage affectent la qualité des eaux souterraines, et la production et l'application d'engrais azotés entraîne des émissions de gaz à effet de serre (GES) contribuant au réchauffement climatique. En plus, les fluctuations du coût de l'énergie, l'accroissement des réglementations communautaires et des pressions environnementales conduisent au développement de systèmes de culture à bas niveaux d'intrants, plus économes en azote. Ce défi se pose particulièrement chez le colza qui est la principale culture oléagineuse en Europe et la troisième au niveau mondial. Or, la culture de colza est très dépendante de l'alimentation azotée avec environ 65 kg d'azote nécessaires par tonne de grains récoltés (contre 30 kg t⁻¹ pour le blé). La création de nouvelles variétés avec un rendement élevé et stable dans des conditions à plus faible disponibilité en azote (N) devient donc un enjeu majeur, pour lequel l'amélioration de la l'efficacité d'utilisation de l'azote (NUE, N-Use Efficiency) pourrait être un facteur de réussite. Ceci impose une connaissance approfondie de la variabilité génétique des processus sous-tendant la NUE, notamment dans des conditions à faible disponibilité en azote.

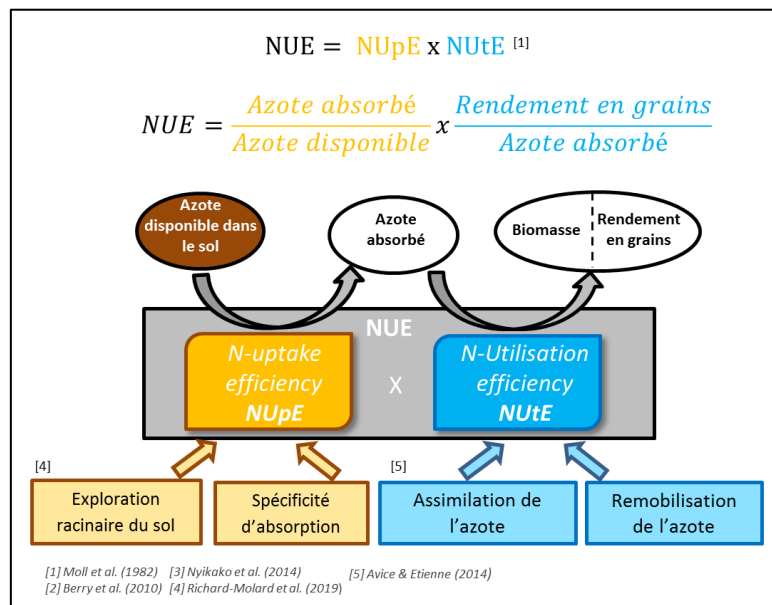
Les réponses du colza à la disponibilité de l'azote résultent des processus complexes qui sont en interaction tout au long du cycle de la plante et qui présentent une grande variabilité génétique et environnementale. Ainsi, l'identification des traits pertinents pour la sélection nécessite une meilleure compréhension du fonctionnement de cette plante en réponse à la disponibilité en azote. Les multiples processus et caractéristiques liés à la réponse de la plante à une faible disponibilité de l'azote nécessitent d'identifier et hiérarchiser des variables permettant de déchiffrer le processus écophysiological déterminant l'élaboration de la biomasse tout au long du cycle.

La NUE dépend elle-même de l'efficacité d'absorption de l'azote (NUpE, Nitrogen Uptake Efficiency) et l'efficacité d'utilisation (pertes et recyclage) de l'azote (NUtE, Nitrogen Utilisation Efficiency). L'optimisation de l'acquisition des ressources azotées (NUpE) mais aussi carbonées pendant la phase végétative est un levier d'amélioration de la NUE encore peu étudié. Pourtant des travaux ont montré que la quantité d'azote absorbée avait un effet important sur l'établissement de l'indice foliaire qui lui-même influence l'élaboration du nombre de grain au m².

L'amélioration de la NUE nécessite donc une connaissance approfondie des traits en lien avec le développement et la croissance des organes racinaires et aériens, ainsi qu'une étude de leur variabilité génétique. En effet l'absorption d'azote dépend d'une part des capacités d'absorption de chaque génotype (NUpE) mais aussi du volume de sol exploré et de la surface absorbante, dépendante de la taille et de la densité des racines. Des travaux

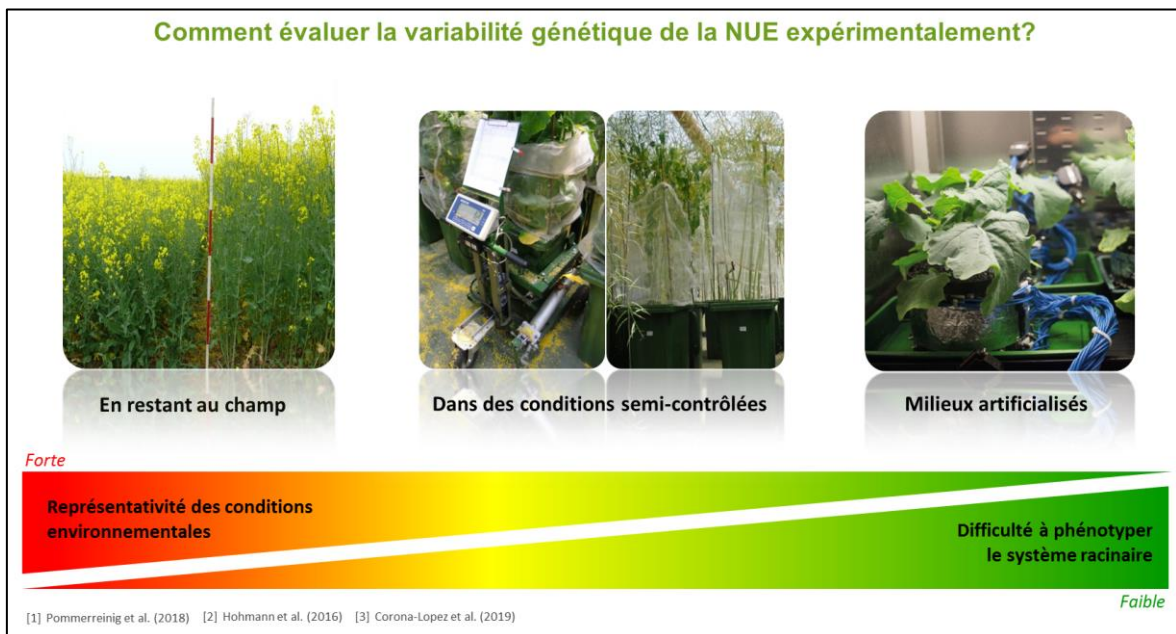
ont mesuré la NUpE chez le colza à la récolte des graines, et ont conclu que cette composante de la NUE serait principal facteur limitant la NUE dans des conditions N limitantes. La variabilité génotypique de la NUpE pourrait avoir un impact précoce sur l'élaboration de la biomasse. Il y aurait donc un intérêt de déchiffrer la dynamique des liens entre NUpE et NUtE au cours du cycle du colza.

Évaluer la variabilité génétique de la NUE en conditions agronomiques et comprendre l'établissement de la biomasse en réponse à l'azote nécessite de i) phénotyper de la biomasse totale produite au cours du cycle : difficulté inhérente de phénotyper les racines ; ii) caractériser l'état de l'azote de la plante, et iii) estimer précisément l'azote disponible dans le sol. Il est donc nécessaire de mieux connaître le fonctionnement des racines en particulier des racines fines qui représentent une petite biomasse mais font l'essentiel du prélèvement de l'azote. La caractérisation des réponses du colza à la disponibilité de l'azote nécessite un phénotypage précis, à haut débit, des variables d'intérêt et des dispositifs expérimentaux adaptés à la conduite



des plantes tout au long du cycle, ainsi qu'une caractérisation précise de l'état azoté de la plante ainsi que de la quantité d'azote disponible dans le sol, notamment dans des situations à faible disponibilité en azote. En effet, la portée de l'amélioration de la NUE a été limitée en raison de la difficulté inhérente au phénotypage de l'ensemble du système végétal, y compris les racines.

Pour décrypter l'adaptation des plantes à la disponibilité de l'azote, les processus d'interaction impliqués dans le fonctionnement de l'azote doivent être considérés à l'échelle de la plante entière en intégrant la dynamique source-puits et la plasticité architecturale des cultures en peuplement. Ainsi il est nécessaire i) de disposer des variables clé acquises en contexte de peuplement (à cause de la plasticité des plantes, impossible d'étudier ces processus sur plantes individuelles) ; ii) être capable de phénotyper à différents moments du cycle et jusqu'à la récolte finale pour faire le lien avec les variables de sorties (rendement et NUE finale à la récolte) ; et iii) accéder à tous les compartiments contributeurs impliqués dans l'acquisition N et C (surfaces d'échanges, compartiment d'allocation/de remobilisation du C et N).



Cette forte interaction entre les processus contribuant à la NUE et l'acquisition du C/N au cours du cycle limite l'étude via le phénotypage des variables d'état. Ainsi, un phénotypage assisté par modèle permettrait d'étudier des paramètres qui régissent les relations entre variables et donc i) tenir compte des relations entre variables : causalité vs corrélation ; ii) intégrer l'aspect dynamique de l'élaboration des variables au cours du cycle ; iii) s'abstraire du bruit environnemental ; et iv) hiérarchiser des paramètres pertinents pour la sélection. Cependant, il reste à développer des modèles écophysiologicals qui rendent compte du fonctionnement C-N à l'échelle de la plante entière et qui rendent compte de la variabilité génotypique de la NUE en réponse à l'azote.

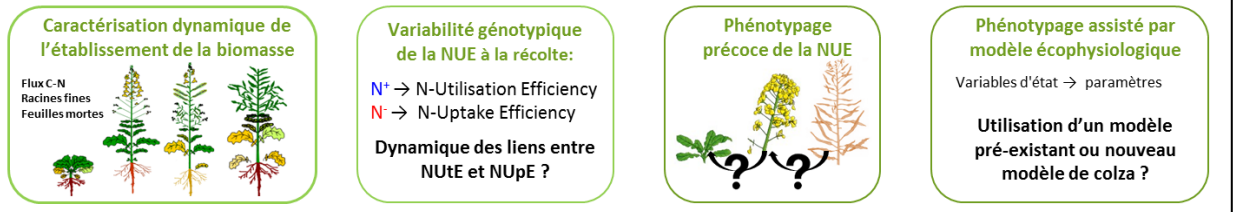
De la problématique à la question de recherche :

Réduire l'apport d'intrants azotés pour une agriculture plus durable

- En agissant sur le sol + pratiques culturales adaptées
- **Identifier des variétés plus efficaces:** meilleur ratio rendement/N disponible



Améliorer la NUE du colza + identifier des traits de sélection avant la récolte



Cette thèse vise à répondre la question de recherche suivante :

Quels sont les principaux processus écophysologiques et traits sous-tendant la NUE à différents stades de développement et qui soient de bons indicateurs de la variation génotypique du colza en réponse à l'azote ?

Cette question a été décomposée en trois sous questions :

- Quel indicateur précoce pour représenter la variation génotypique de la NUE en réponse à l'azote à la récolte ?
- Quels sont les processus racinaires qui expliquent précocement la variabilité génétique de la NUE ?
- Quels sont les principaux traits à l'échelle de la plante entière qui déterminent l'accumulation de biomasse et les flux de C-N entre les organes, qui eux-mêmes sous-tendent la NUE ?

Pour y répondre, nous avons choisi une approche combinant un phénotypage expérimental sur le terrain et un phénotypage assisté par modèle. Nous avons choisi un dispositif de culture adapté appelé PERISCOPE, (Bissuel-Belaygue et al., 2015). Dans nos expérimentations, la réponse des plantes à la disponibilité en azote a été étudiée en évaluant des variables fonctionnelles liées à l'azote tout au long du cycle de culture (c'est-à-dire la matière sèche totale du compartiment aérien et racinaire et à leur teneur en C et en azote) et des variables intégratives telles que le rendement des grains, évaluées à la maturité des graines. Pour l'approche de phénotypage assisté par modèle, nous avons proposé un cadre conceptuel décrivant le fonctionnement C et N du colza d'hiver, y compris les racines fines, la chute des feuilles et l'émergence des tiges.

MISE EN PLACE DES EXPERIMENTATIONS

Trois expérimentations ont été menées sur deux sites différents et sur deux années climatiques, au Rheu en 2014-2015 et à Grignon en 2014-2015 et 2017-2018, ci-après dénommées respectivement LR15, GR15 et GR18. Les expérimentations de LR15 et GR15 ont été réalisées avant le début de mon doctorat, tandis que j'ai personnellement conçu et réalisé l'expérimentation GR18. Les expérimentations ont été réalisées à l'aide du dispositif PERISCOPE (Bissuel-Belaygue et al., 2015). Un total de huit génotypes de colza d'hiver (*Brassica napus* L.) ont été expérimentés dans des conditions d'azote contrastées. Nous avons pris soin de comparer des génotypes ayant des rendements en graines contrastés au champ mais avec des cycles de croissance et des dates de floraison similaires. Le génotype AVISO a été testé comme témoin dans toutes les expérimentations.

Les génotypes ont été cultivés dans des tubes et ont été regroupés dans des bacs de 1 m³ pour obtenir un peuplement reconstitué avec une densité de 35 plantes m². Dans les bacs, l'espace entre les tubes a été rempli de terre pour assurer l'isolation thermique du système racinaire. En outre, pour éviter les effets de bordure, deux rangées de plantes ont été semées sur chaque bord du bac et une seule plante a été gardée par tube après démariage. Nous avons récolté les plantes à plusieurs stades de développement tout au long du cycle de culture. Les dates de prélèvement varient en fonction de l'expérimentation, mais, ensemble, ont permis d'obtenir un jeu de données complet sur la dynamique de croissance. Pour simuler des conditions constantes de faible et de forte teneur en azote pendant le cycle de croissance de la culture, une solution minérale a été fournie tous les 200 degrés-jours de croissance, de l'émergence à la maturité des graines, soit 13 à 14 applications pendant le cycle de croissance. De plus, nous avons maintenu l'humidité du sol au-dessus de 85 % de la capacité du champ, évitant ainsi tout autre stress non contrôlé (c'est-à-dire le stress hydrique et la perte d'éléments nutritifs par lixiviation).

À chaque date de prélèvement, nous avons divisé les plantes récoltées en fractions : pivot, racines fines, feuilles (vertes, sénescentes et tombées), tige principale, branches et siliques. Des variables en lien avec la partie aérienne (i.e. diamètre de la tige, la hauteur de la plante, le nombre de feuilles, le nombre de branches, le nombre de siliques et de graines) et la partie racinaire (ie. nombre de racines secondaires, la profondeur et la longueur du pivot) ont été mesurées manuellement. La matière sèche et la teneur en C et N des différentes fractions de plantes ont été mesurées, et la surface des feuilles vertes et des siliques a été évaluée. Les horizons du sol ont été échantillonnés pour la caractérisation de l'azote minéral et la quantification de l'humidité du sol. Pour caractériser l'état nutritionnel de la culture, nous avons utilisé l'indice de nutrition azotée (INN). Les caractéristiques spécifiques à chaque expérimentation ont été définies dans le Chapitre I, section 1.2.2.

CHAPITRE II. SYNTHÈSE BIBLIOGRAPHIQUE

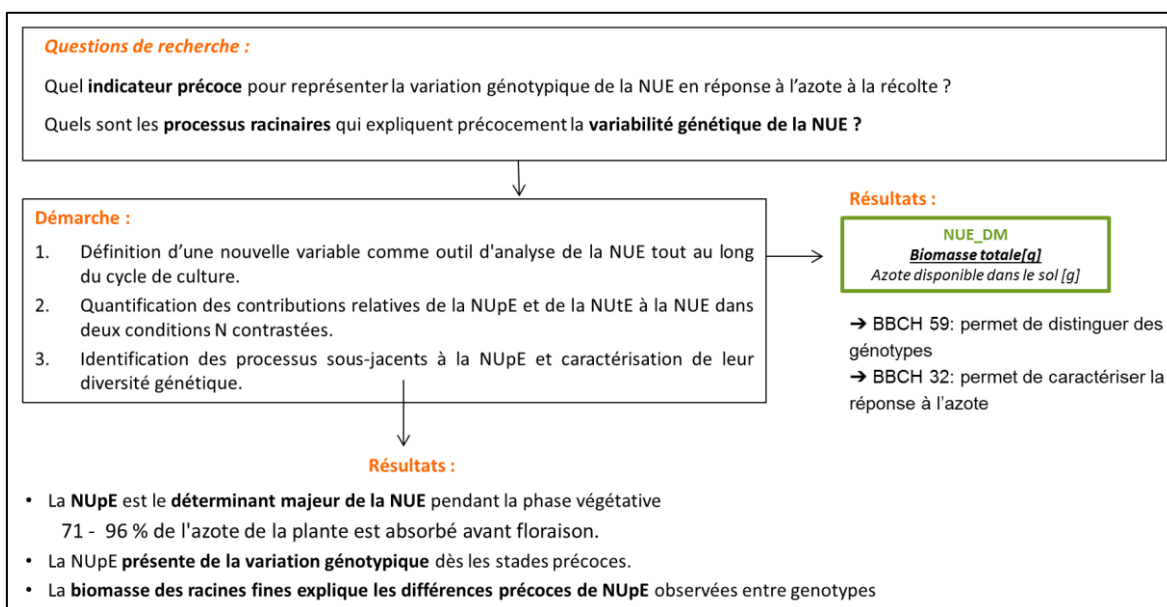
Ce chapitre correspond à une revue bibliographique. Il fait le point sur les connaissances disponibles en lien avec le sujet de la thèse. Ce chapitre met en évidence la nécessité de définir clairement la NUE lorsqu'on discute de son amélioration ou lorsqu'on compare les résultats de différentes études, puisque nous avons recensé neuf définitions liées à l'efficacité d'utilisation de l'azote. De plus, l'estimation de la NUE est confrontée à une quantification précise de l'azote disponible dans le sol pendant le cycle de culture et à la quantification de la quantité totale d'azote accumulée dans les plantes, y compris les feuilles mortes et les racines fines. Dans ce travail de thèse, nous avons défini le NUE comme le rapport entre la matière sèche de la plante entière (incluant les racines fines et les feuilles mortes) et l'azote disponible dans le sol (incluant la fertilisation azotée et l'azote minéral dans le sol). Cette forme de calcul de la NUE est expliquée et détaillée dans le Chapitre III.

Dans ce chapitre, nous suggérons une amélioration potentielle des performances du colza dans un contexte de faibles apports d'azote en améliorant l'efficacité de l'absorption de l'azote (NUpE), mais nous soulignons également que la contribution de la NUpE à la NUE a été peu documentée. Dans cette thèse nous avons étudié les traits liés à la NUpE pendant la phase végétative, permettant une meilleure compréhension de la variabilité génotypique de la NUE tout au long du cycle de la culture dans des conditions de faible teneur en azote (chapitre III). Nous avons souligné que la croissance et le développement du colza sont complexes et que les principales périodes d'accumulation de la biomasse et d'absorption de l'azote se chevauchent au cours du cycle.

Les effets d'une faible disponibilité en azote sont souvent quantifiés à l'échelle du couvert et à maturité des graines, mais une meilleure compréhension des processus sous-jacents à la NUE à l'échelle de la plante est nécessaire, ainsi que leur modulation par une faible disponibilité en azote. En outre, compte tenu de la grande plasticité du développement du colza en réponse aux conditions climatiques, et des capacités de compensation des organes entre la phase végétative et la phase reproductive, les processus à l'échelle de la plante méritent d'être étudiés dans des conditions de terrain ou, du moins, sous des couverts reconstitués à l'échelle du terrain tout au long du cycle de croissance de la culture. Par conséquent, nous avons souligné la nécessité d'une meilleure compréhension de la dynamique des flux de C et de N aux différentes phases de croissance et de développement pour améliorer la NUE chez le colza. En outre, nous avons souligné que des méthodes et des approches de phénotypage adaptées sont nécessaires, notamment en ce qui concerne le phénotypage des racines fines dans des conditions proches de celles du terrain. Dans ce travail de thèse, nous avons conduit trois expérimentations complémentaires en termes d'acquisition des données, à l'aide d'un dispositif permettant cultiver les plantes dans des conditions semi-contrôlées, ce qui a permis l'accès à l'ensemble des organes de la plante tout au long du cycle, y compris les racines fines. Ainsi, nous avons réalisé de multiples prélèvements destructifs tout au long du cycle de culture, permettant de caractériser l'élaboration de la biomasse et la dynamique d'absorption de l'azote depuis le développement des feuilles jusqu'à la maturité des graines (Chapitre I, Figure I.2.).

Enfin, nous avons souligné qu'une approche de phénotypage assistée par modèle pourrait être pertinente pour identifier et hiérarchiser les paramètres de la plante entière qui sous-tendent les variations génétiques de la NUE en réponse à la disponibilité en azote, car ces paramètres sont interconnectés et soumis à des nombreuses rétroactions. Cependant, les modèles permettant une description de la plante entière sur l'ensemble du cycle de culture restent rares pour le colza et il n'existe pas de modèle décrivant les flux de N et de C en considérant les parties aériennes ainsi que les racinaires. Néanmoins, une telle approche a été utilisée avec succès, par exemple, pour analyser la variabilité génotypique des traits de la plante entière associés à l'absorption de N et à la NUE en réponse à une carence en N chez le blé (Laperche et al., 2006) et chez *Arabidopsis thaliana* (Richard-Molard et al., 2009). Par conséquent, nous avons proposé un cadre conceptuel pour modéliser le fonctionnement C-N du colza au niveau de la plante entière, y compris les compartiments aériens et racinaires, et nous l'avons utilisé pour identifier les traits de la plante entière expliquant l'élaboration de la biomasse en réponse aux conditions d'azote et sa variation génotypique dans des conditions de faible teneur en azote (chapitre IV).

CHAPITRE III. Quel indicateur précoce pour représenter la variation génotypique de la NUE en réponse à l'azote à la récolte ? Quels sont les processus racinaires qui expliquent précocement la variabilité génétique de la NUE ?



Dans le chapitre II, nous avons identifié une marge d'amélioration de la NUpE, qui pourrait être à l'origine des différences génotypiques observées dans la NUE à la récolte, notamment dans des conditions de faible disponibilité en azote. Cependant, nous avons souligné une carence dans les méthodes de phénotypage permettant de caractériser et de quantifier les processus liés à la NUE, y compris les feuilles mortes et les racines fines. En effet, l'analyse dynamique des contributions de NUpE et NUtE aux variations du NUE fait encore défaut dans la littérature.

L'objectif de ce chapitre est (i) de quantifier dynamiquement les contributions relatives des composantes NUpE et NUtE aux variations temporelles et génotypiques de la NUE tout au long du cycle de culture du colza d'hiver dans deux conditions d'azote contrastées ; et (ii) de découvrir les processus sous-jacents à la variabilité génotypique de la NUpE. En effet, la composante NUpE pourrait devenir le principal facteur limitant la NUE dans le contexte émergent de la réduction des apports d'azote, mais son rôle dans le déterminisme de la NUE est encore peu documenté.

Nous avons procédé en deux étapes. Premièrement, nous avons étudié une nouvelle variable liée à la NUE comme outil d'analyse de la NUE tout au long du cycle de culture. Deuxièmement, nous avons quantifié l'impact de la NUpE, considérée comme un processus dynamique, sur la NUE et avons décodé les contributions relatives de la NUpE et de la NUtE à la NUE dans deux conditions d'azote contrastées. Troisièmement, nous nous sommes concentrés sur les sous-processus sous-jacents à la NUpE (c'est-à-dire l'absorption spécifique de N et la croissance des racines) et avons caractérisé leur diversité génétique dans un ensemble de génotypes représentant le matériel génétique du colza d'hiver.

Nous avons sélectionné des génotypes de colza d'hiver contrastés en termes de variation du rendement en graines aux apports d'azote, et nous avons analysé la cinétique de la croissance, la biomasse et la teneur en azote des pousses et des racines sur l'ensemble du cycle végétatif. Étant donné que la NUE mesurée à la récolte des graines est une variable intégrative de la croissance de la plante, de la disponibilité de l'azote et de l'élaboration du rendement au cours du cycle de culture, nous avons calculé une NUE plus précoce aux principaux stades du cycle de croissance, en tenant compte de la biomasse de la plante entière produite depuis le semis (NUE_DM).

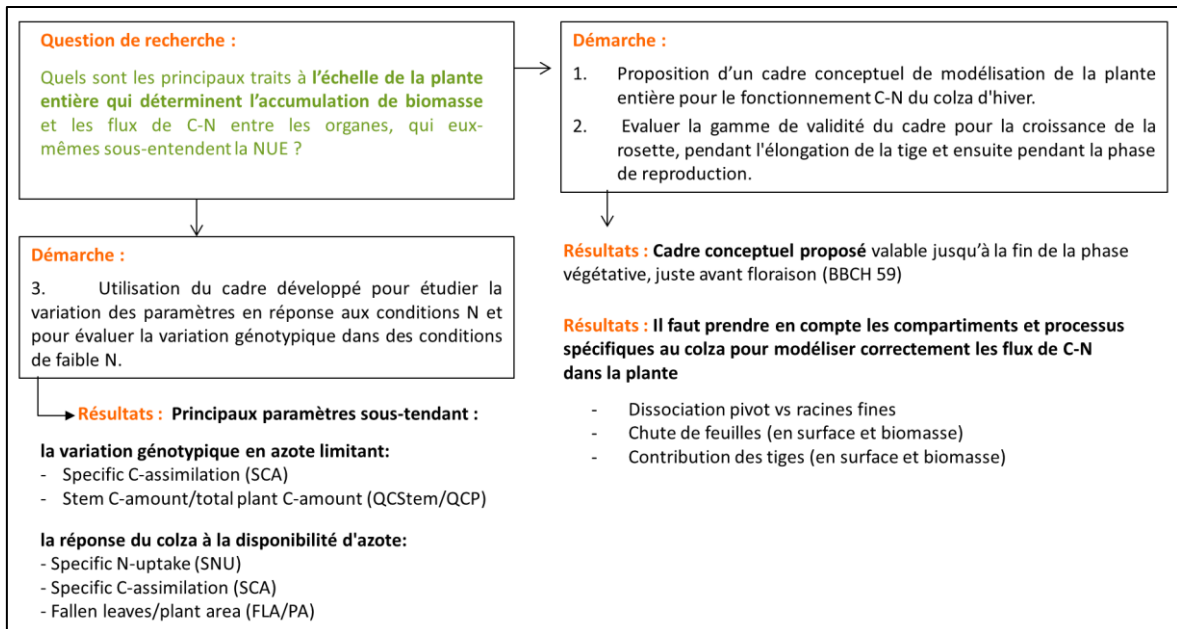
Étant donné que nous avons déterminé une forte corrélation entre la NUE défini comme le rendement en graines par unité d'azote du sol disponible à la récolte et la NUE_DM à la récolte, nous avons étudié la qualité de cette corrélation à différents stades antérieurs du cycle de culture. Nous avons examiné le stade le plus précoce dans lequel la NUE_DM était corrélée avec le point de récolte. Nous avons démontré que la NUE_DM mesurée au début de l'élongation de la tige (BBCH 32) pouvait être utilisée comme un trait de substitution précoce et robuste pour caractériser la réponse à l'azote à la récolte. Cependant, pour discriminer les génotypes, la NUE_DM devrait être mesurée dès la fin de la phase végétative (BBCH 59). Nous avons montré que la variation de la NUpE explique mieux la variation de la NUE_DM que la NUtE pendant la croissance d'automne quelque soit la condition azotée. Cependant, nous avons observé un changement dans les contributions de la NUpE et de la NUtE à la NUE_DM à partir de la floraison, mais avec des tendances différentes selon les conditions d'azote. Alors que dans des conditions d'azote non limitante la NUtE a principalement contribué à la variation de la NUE_DM pendant la phase de reproduction, la NUpE y contribue principalement dans des conditions d'azote limitantes. Néanmoins, dans des conditions d'azote non limitantes, la contribution de la NUpE était toujours importante et ne devrait pas être négligée.

Nous avons étudié la variation génotypique de la NUE_DM et de ses composantes, et nous avons mis en évidence un effet génotypique plus élevé pendant la phase végétative dans des conditions d'azote limitantes. En conséquence, la NUpE a été identifiée comme étant le principal

facteur de variation de la NUE_DM. Nous avons mis en évidence que la NupE était le principal moteur de la variabilité génotypique de la NUE_DM pendant la croissance automnale, ce qui nous a conduit à évaluer les processus clés qui sous-tendent ce trait et leur variabilité génotypique. La variabilité génotypique de la NUpE n'était pas due à l'absorption spécifique de l'azote (SNU) mais à la croissance des racines fines. L'accumulation de la biomasse des racines fines au cours du cycle de croissance était positivement corrélée avec la NUpE, ce qui a mis en évidence la biomasse cumulée des racines fines comme un trait précoce déterminant l'efficacité d'utilisation de l'azote.

Nos résultats ont suggéré un écart entre, d'une part, la capacité de la NUE_DM à prédire les variations génotypiques de la NUE à la maturité des graines (valable à partir de BBCH 59) et, d'autre part, le rôle de la NUpE et de la croissance des racines fines comme principaux déterminants de la NUE_DM (valable pendant la croissance en automne). Ainsi, pour optimiser pleinement la performance génotypique en termes de la NUE, il serait nécessaire de mieux comprendre les changements qui se produisent dans la plante pendant l'élongation de la tige (période d'écart). Une approche de modélisation pourrait être utile pour comprendre et classer l'importance des processus liés à l'acquisition et au partage du C-N, qui sont fortement modifiés par le développement de nouveaux organes durant cette période.

CHAPITRE IV. Quels sont les principaux traits à l'échelle de la plante entière qui déterminent l'accumulation de biomasse et les flux de C-N entre les organes, qui eux-mêmes sous-entendent la NUE ?



Dans le chapitre III, nous avons mis en évidence la NUE_DM comme un proxy de la NUE_Seed, permettant de discriminer les génotypes à partir de BBCH 59 et les effets de l'azote à partir de BBCH 32. La NUE_DM repose directement sur l'élaboration de la biomasse de la plante

entière et le chapitre II a mis en évidence que les processus conduisant à l'élaboration de la biomasse totale en réponse aux conditions N sont régulés par les flux de C et N dans la plante entière. Par conséquent, nous avons suggéré que l'efficacité de l'utilisation de l'azote pourrait être évaluée dès la phase végétative en utilisant un cadre conceptuel du fonctionnement C-N de la plante entière qui rend compte de la dynamique de l'élaboration de la biomasse au cours du cycle de croissance.

Dans le chapitre III, nous avons mis en évidence que la dynamique de l'établissement des racines fines était le principal moteur des différences génotypiques observées dans la NUpE pendant la croissance d'automne. Cependant, le phénotypage de la variation génotypique de la NUpE et de la croissance des racines fines reste délicat, surtout en conditions au champ. Une approche de phénotypage assistée par modèle pourrait permettre de mieux comprendre la répartition de la biomasse entre les compartiments de la pousse et de la racine en réponse à l'azote et de simuler le génotype et la biomasse des racines fines plutôt que de la mesurer.

Ce chapitre IV porte sur le développement et la validation d'une approche écophysologique basée sur la modélisation pour identifier les processus principaux contribuant à la NUE, et les traits impliqués dans la tolérance du colza à une faible disponibilité en azote. Un point fort du cadre conceptuel développé est qu'il décrit les interactions C et N à l'échelle de la plante entière en rendant compte de la dynamique de l'élaboration de la biomasse pendant le cycle de croissance et en tenant compte des spécificités du colza d'hiver. En intégrant des pools de biomasse souvent négligés (feuilles mortes, tiges, racines fines) et variables nouvelles telles que la surface des feuilles mortes de la plante ou la biomasse en racines fines, le modèle a permis de mieux comprendre la répartition de la biomasse dans les compartiments aérien et racinaire en réponse à l'azote et au génotype.

Cette analyse a été décomposée en trois étapes. Premièrement, nous avons proposé un cadre de modélisation conceptuelle de la plante entière du fonctionnement C-N du colza d'hiver. Deuxièmement, nous avons évalué la validité des relations qui ont été ajustées pour la croissance de la rosette, pendant l'élongation de la tige et ensuite pendant la phase reproductive. Troisièmement, nous avons utilisé le cadre développé pour étudier la variation des paramètres en réponse aux conditions d'azote et pour évaluer la variation génotypique dans des conditions de faible teneur en azote. Un tel cadre peut-il être utilisé pour réduire l'étendue de la variation génotypique dans les processus sous-jacents à la production de biomasse en réponse aux niveaux de nutrition N ?

Nous avons proposé un cadre de modélisation conceptuel de la plante entière adaptée au colza d'hiver afin d'expliquer les flux de C et de N entre les organes de la plante pendant la période végétative. Nous l'avons utilisé i) pour évaluer les processus de la plante entière soutenant la variation génotypique observée en réponse aux conditions d'azote, et ii) pour réduire et hiérarchiser la source de variation génotypique des réponses de la plante en condition de faible disponibilité en azote à un nombre limité de traits sous-jacents à l'accumulation de biomasse. Considérant que la NUE peut être exprimée comme le rapport entre la biomasse produite et l'azote disponible, un modèle qui rend compte de la dynamique de l'accumulation de la biomasse de la plante entière au cours du cycle de croissance pourrait être utilisé pour calculer la NUE.

Ce cadre conceptuel de modélisation s'est avéré être un outil pertinent pour comprendre la réponse contrastée à l'azote. L'évaluation de la variation génotypique dans des conditions à faible disponibilité en azote par le biais des paramètres du modèle semble être plus pertinente que l'étude des variables d'état en telles qu'elles, mesurées ponctuellement au cours du cycle de croissance de la plante, telles que la biomasse, les surfaces ou les teneurs spécifiques en C et en N.

Nos résultats ont montré que la réponse du colza d'hiver à la disponibilité en azote était principalement soutenue par l'efficacité de l'assimilation du carbone (SCA), l'absorption spécifique de l'azote (SNU) et la part des feuilles mortes par rapport à la surface de la plante (FLA/PA). En revanche, la variation génotypique dans des conditions de faible disponibilité en azote était soutenue par la SCA, la répartition du carbone entre les feuilles et les tiges, et le rapport entre les racines fines (FineR/RDM). Comme aucune différence n'a été observée pour le SNU, nous avons suggéré que le rapport des racines fines est le principal paramètre qui sous-tend la variation génotypique de l'efficacité d'absorption de l'azote. En conclusion, l'analyse conduite a permis de faire apparaître le rôle de l'assimilation spécifique de carbone, la part de carbone allouée aux tiges et la proportion de racines fines comme paramètres clés de la réponse génotypique à l'azote, et de suggérer que la NUpE et la proportion de racines fines pourraient être des indicateurs de la NUE pour cribler précocement les variétés colza d'hiver pendant la phase végétative.

La prochaine étape consistera à mettre en œuvre le cadre conceptuel de modélisation proposé et à évaluer la sensibilité de la biomasse végétale et de ses composantes aux paramètres ciblés. Le modèle pourrait alors être utilisé pour le phénotypage à haut débit à des stades précoces et pour évaluer davantage l'impact de la croissance des racines sur les processus précoces liés à la NUE.

CHAPITRE V DISCUSSION ET PERSPECTIVES DE RECHERCHE

Ce travail de thèse, basé sur une combinaison d'approches expérimentales et d'un phénotypage assisté par la modélisation, apporte des résultats significatifs et des connaissances physiologiques nouvelles sur l'efficacité de l'utilisation de l'azote, un processus constituant une question essentielle pour assurer la durabilité et la compétitivité du colza d'hiver. Ce travail de thèse a permis d'identifier les processus principaux contribuant à l'efficacité d'utilisation de l'azote et les traits impliqués précocement dans la tolérance du colza à une faible disponibilité en azote et sa variabilité génotypique. Il permet de proposer des leviers physiologiques pour l'amélioration des variétés de colza dans un contexte agro-écologique de réduction des apports en azote.

Appréhender la NUE comme le ratio entre de la biomasse totale produite par la quantité d'azote disponible dans le sol (NUE_DM) a permis d'identifier les processus physiologiques à l'origine des différences finales de rendement en grains à la récolte. La NUE_DM a l'avantage

d'être un indicateur quantifiable à n'importe quel stade phénologique et pourrait être utilisée comme un est un indicateur précoce de la NUE_seed à la récolte, capable de discriminer les géotypes dès BBCH 59 (juste avant floraison) et de caractériser la réponse à l'azote dès BBCH 32 (l'élongation de la tige). L'exclusion des stades plus précoces (*i.e.* BBCH16-19) dans l'approche expérimentale proposée peut être un inconvénient pour le phénotypage précoce, car les plateformes de phénotypage à haut débit limitent souvent les recherches sur les colzas au début du développement de la plante, d'où l'intérêt du phénotypage assisté par modèle.

La NUpE est un processus qui mérite d'être étudié en profondeur. Nous avons mis en évidence le rôle majeur de la NUpE dans la détermination de la NUE dès les stades de développement précoces et quelle que soit la condition d'azote. La variabilité génotypique de la NUpE ne dépend pas de l'absorption spécifique d'azote (SNU) ; dépend de la croissance des racines fines. Il est donc nécessaire de phénotyper les racines fines car la biomasse des racines fines explique jusqu'à 92% de la variation génotypique dans des conditions de faible azote. L'émergence des racines fines comme levier prometteur de l'amélioration génotypique NUE soulève également de nouvelles questions pour les méthodes de phénotypage des racines. En plus, L'identification du SCA comme un paramètre sous-portant la réponse génotypique à l'azote souligne la nécessité de prendre en compte l'allocation de carbone et azote au niveau de la plante entière. Ce paramètre détermine la quantité d'azote totale de la plante et donc le réservoir potentiel qui pourra être alloué aux racines.

Cette thèse a mis en évidence plusieurs perspectives de recherche :

- **Implémenter le modèle pour simuler l'élaboration de la biomasse et conduire des analyses de sensibilité sur les paramètres :**

Caractériser les liens entre paramètres d'efficacité et la NUpE, NUtE et NUE

- **Tester un plus grand nombre de géotypes** présentant des morphologies racinaires contrastées : renforcer la relation entre l'architecture racinaire et les NUpE.
- **Changement d'échelle et expérimentation au champ** : forte plasticité du colza: les processus alliant un impact sur les flux de C-N et leur mise en réserve temporaire méritent d'être étudiés dans des conditions agronomiques au champ
- **Evaluer l'impact de combinaison de stress et des nouvelles systèmes et pratiques agricoles sur les flux de C-N** : compréhension approfondie de ces systèmes complexes (*i.e.* interaction entre disponibilité N et stress hydrique, synergie/compétition en association de cultures, nouvelles pratiques de fertilisation – organique, foliaire-)

*“Caminante, son tus huellas
el camino y nada más;
Caminante, no hay camino,
se hace camino al andar.
Al andar se hace el camino,
y al volver la vista atrás
se ve la senda que nunca
se ha de volver a pisar.
Caminante, no hay camino
sino estelas en la mar.”*

– Antonio Machado

Give me wings to fly, roots to come back, and reasons to stay.

– Dalai Lama

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GENERAL INTRODUCTION

Toward oilseed rape varieties for sustainable production

Nitrogen (N) fertilization, coupled with the genetic progress, has enabled an increase of crop yields for several decades. However, excessive application of N fertilizers can be a major environmental problem, affecting groundwater quality through N leaching and contributing to global warming through greenhouse gas (GHG) emissions from production to application. Moreover, the increased competition for nonrenewable fossil fuel reserves has directly elevated prices of N fertilizers and the cost of agricultural production worldwide. In addition, the substitution of mineral N fertilizers by organic fertilizers generates more fluctuation in crop N-availability. These factors pose a considerable challenge in the quest to breed N-efficient crops—that is, crops with high and stable yields under low-N inputs.

This challenge is of particular importance for vegetable oil production. Oilseed rape, one of the main oilseed crops in Europe and the major one in France, could partly meet this challenge in terms of seed oil content and quality. However, oilseed rape is highly dependent on N fertilization and apparently presents a non optimal use of N-fertilization, *i.e.* low N-use efficiency (50 kg N ha⁻¹ to produce 1 t ha⁻¹ of seeds). In addition, excessive N fertilization can also reduce the ratio of seed oil content to protein content and increase the plant's susceptibility to disease. Therefore, breeding winter oilseed rape cultivars with increased N-use efficiency constitutes a core issue in ensuring the sustainability and competitiveness of this crop. From a biological point of view, this apparent plant's inefficiency regarding nitrogen is also a scientific issue and makes oilseed rape an interesting model to better understand the complex C-N functioning of crops and explore the genetic variability of the involved processes.

Crossing these societal issues and the scientific ones leads to the challenging research problem of this Ph.D. work.

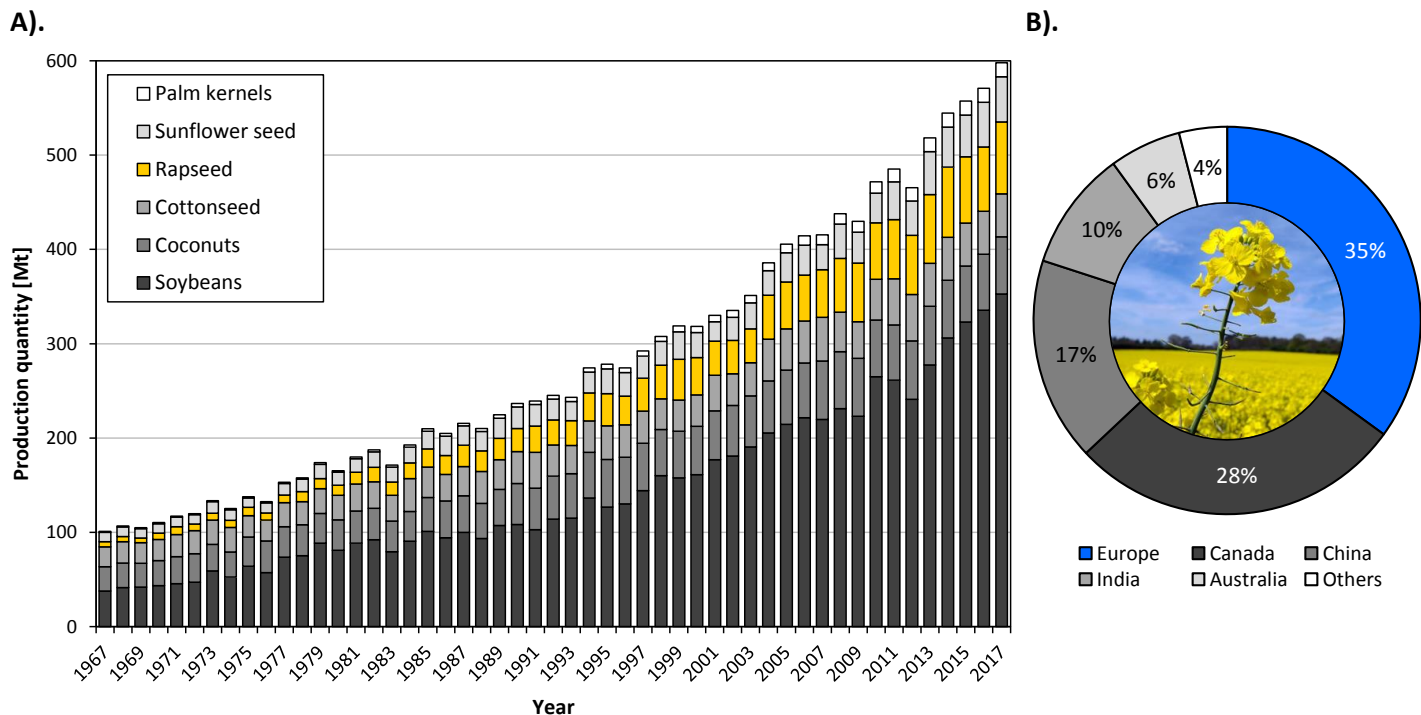


Figure 0.1. A). World oilseed production of the main oilseed crops over the last 20 years. Production includes the quantities of the commodity sold in the market (marketed production) and the quantities consumed or used by the producers (auto-consumption). **B). Main oilseed rape producing countries in 2017.** Diagrams based on the FAOSTAT data consulted in November 2019.

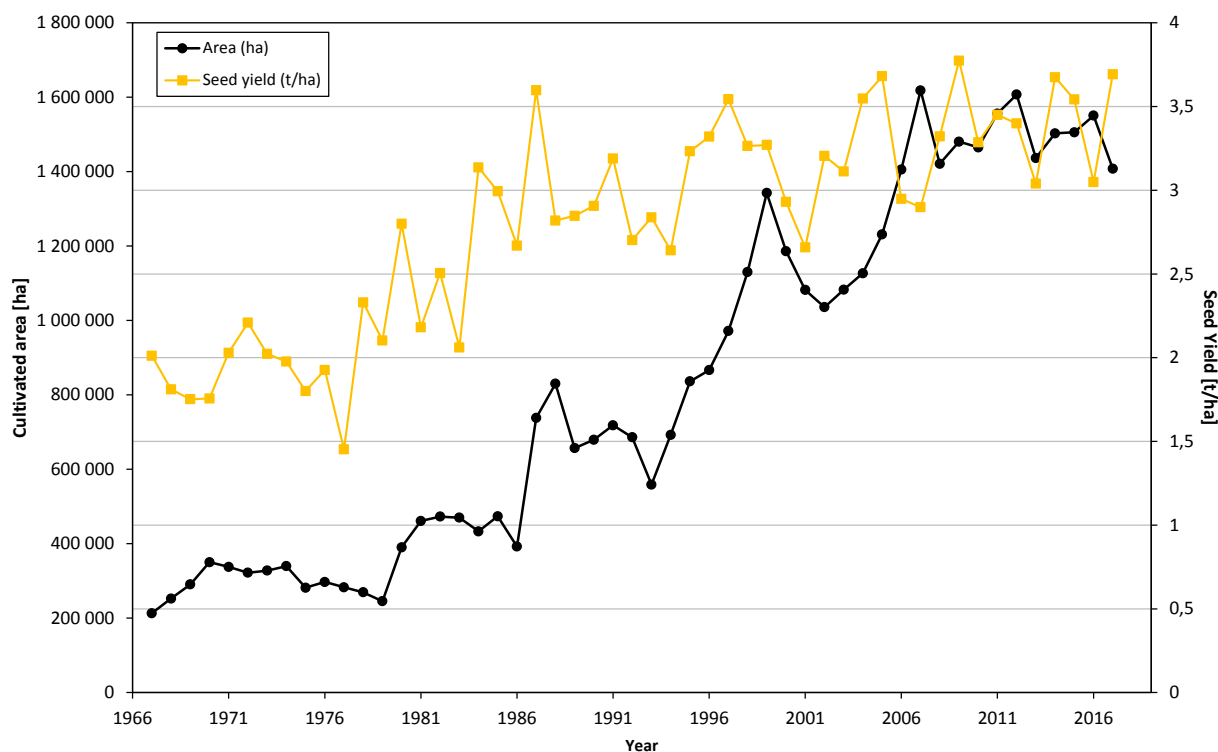


Figure 0.2. Oilseed rape area (in black) and seed yield (in yellow) in France over the last 20 years. Data refers to the harvested area. The diagrams are based on the FAOSTAT data consulted in November 2019.

I. Societal issues

I.1. Importance and utilization of winter oilseed rape

Oilseed rape (*Brassica napus* L.) is currently the second most important oilseed crop in the world after soybeans (Figure 0.1A), with major areas of production in Europe (26.3 Mt), Canada (21.3 Mt) and China (13.3 Mt) (Figure 0.1B) (FAOSTAT, 2017). To date, oilseed rape oil accounts for around 15% of worldwide vegetal oil production. France is the leading oilseed rape producer in Europe (FAOSTAT, 2017) and the second leading exporter worldwide.

In France, oilseed rape is the second most important agricultural crop, behind cereals, in terms of cultivated area (FAOSTAT, 2018). French oilseed rape production is predominantly ensured by winter oilseed varieties, which exhibit an average seed yield from 3 to 5 t ha⁻¹ (at EU level) (Bus et al., 2011), that is about twice that of spring varieties. Oilseed rape has an oil content of 40%–45% of the seed dry matter (Bouchet et al., 2014) and produces around 1,500 L ha⁻¹ of oil. For years, the overall oilseed rape production increase has been mainly due to an increase in cultivated area rather than in seed yield (Sabreena A. Wani, 2018). In the last decade, the area cultivated stagnated in France, and yields remained steady at around 3–3.7 t ha⁻¹ (Figure 0.2), partly due to the effect of climatic change and disease and pest occurrence, that compensated the genetic progress. Indeed, constraints in achieving yield stabilization persist under both high climate variations and biotic and abiotic stresses (Weymann et al., 2015).

Rapeseed oil is primarily used for food and feed and has recently gained increasing attention as a source oil for biodiesel (Berrocoso et al., 2015), particularly in Europe, where the compulsory addition of biodiesel to fossil diesel has created by far the most relevant market for biodiesel. In 2016, according to OilWorld (an independent market analysis publication working on vegetable oils), oilseed rape oil accounted for 58.5 % of Europe feedstock vegetable-oil biodiesel, followed by palm oil (33.2%) and soybean oil (5.9%). European biodiesel represented 44.7% of the world's production in 2016, according to the US Energy Information Administration. Moreover, oilseed rape cultivation has a positive influence on yields of subsequent cereals such as wheat (Christen et al., 1992) and barley (Christen and Sieling, 1993), and therefore an agronomic value in crop rotations. It is considered as a good N catch crop, as it can take up around 100 kg N ha⁻¹ before winter (Sieling et al., 2010), thus reducing nitrate leaching during autumn and winter (Dejoux et al., 2000; Dresbøll et al., 2016).

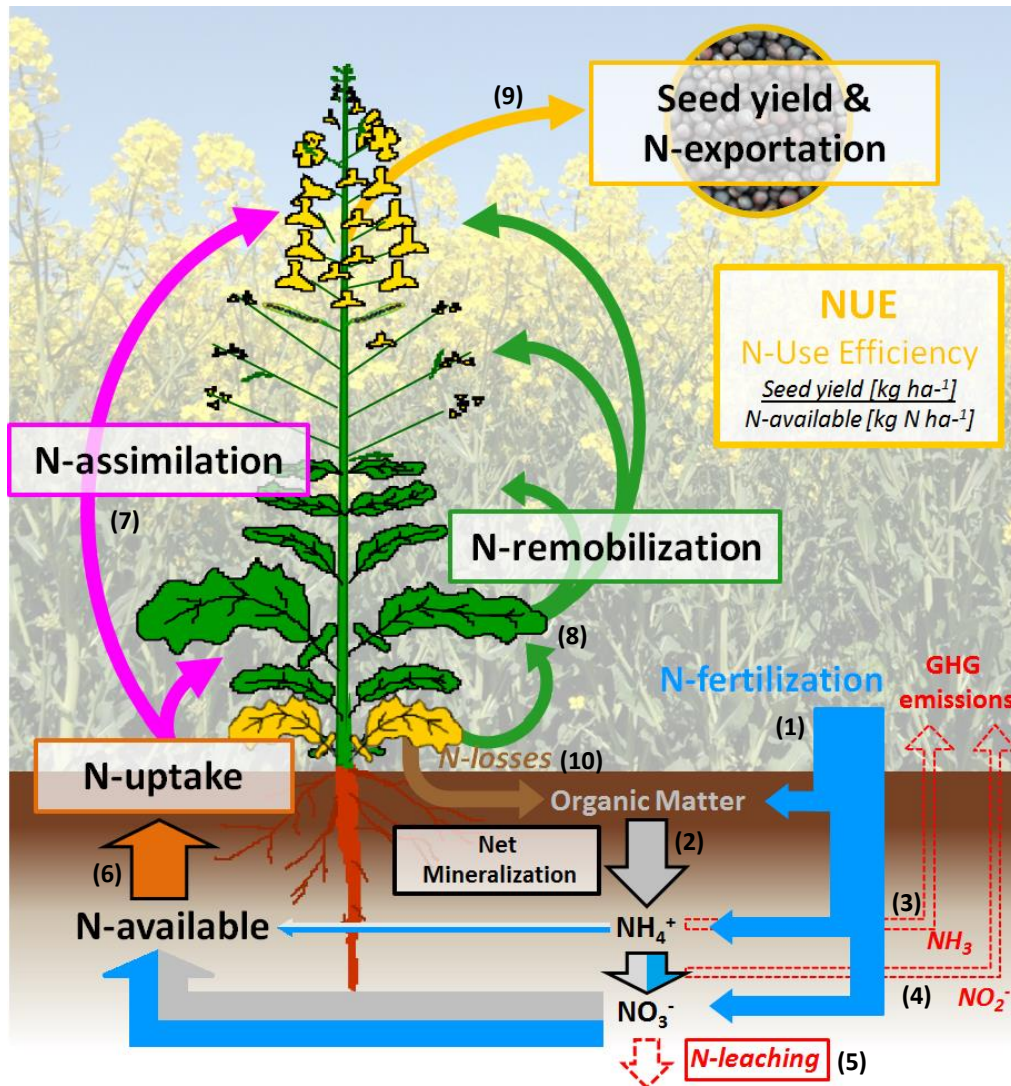


Figure 0.3. Schematic representation of N-fluxes in cropping soil-plant system, determining the N-available in the soil and NUE associated processes (N-uptake, N-assimilation and N-remobilization) at the whole-plant level in oilseed rape. The main N-inputs of the system are N-fertilization (1) and net mineralization of organic matter (2), whereas N-outputs are gaz losses from volatilisation (3) and denitrification (4), nitrate leaching (5) and plant N-uptake (6). N-availability is determined by N-inputs minus N-outputs. Crop NUE (Seed yield per unit of soil N-available) can be subdivided into two main components: N-uptake efficiency (primary fluxes) and N-utilization efficiency (secondary fluxes) that includes N-assimilation efficiency (7) and N-remobilization efficiency (8). Seed yield elaboration and N-exportations (9) result from the interaction of these processes during the whole crop cycle and are impacted by N-availability. N-losses and plant N recycling through leaves losses (10) are not negligible in oilseed rape.

I.2. Nitrogen fertilization and environmental impacts of oilseed rape: breeding for varieties adapted to low N-inputs

World consumption of the three main fertilizing nutrients, nitrogen (N), phosphorus (P_2O_5), and potassium (K_2O), was estimated to reach 186.67 Mt in 2016, up by 1.4% over 2015 consumption levels (FAOSTAT, 2017). A vast amount of N fertilizers is applied to crops worldwide each year. While in 1996, 54.6 Mt of N fertilizers were applied globally, by 2017 it had increased to 109.14 Mt (FAOSTAT, 2017). Although N fertilizers are high energy-intensive and represent the highest input cost for oilseed crops (Rothstein, 2007; Kant et al., 2011), oilseed rape production has increased primarily due to an increase in N fertilizer application (Berry and Spink, 2006). Reducing N inputs while maintaining high yields is therefore essential to ensure a more sustainable and competitive agriculture (Good et al., 2004; Rathke et al., 2006). Moreover, current European regulations tend to reduce the use of N fertilizers (Nitrate Directive, 91/676/CEE), which will require major changes in agricultural practices. Moreover, the increasing environmental concern of consumers has led to the development of new agricultural practices, like organic farming, which require cultivar adapted to lower and more fluctuating N conditions.

Nitrogen-related processes in plant and soil play a key role (Figure 0.3) in biomass production and seed yield elaboration (Crawford and Glass 1998; Hirel et al 2007; Kiba et al., 2016). Oilseed rape requires high N inputs, with 70 kg N ha⁻¹ to produce 1 t ha⁻¹ of seeds (Simonin, 2017), and on average only 50% of the total N applied is exported in seeds (Schjoerring et al., 1995; Rathke et al., 2006), which is around half than for cereals (Sylvester-Bradley and Kindred, 2009). Therefore, oilseed rape is characterized as a crop having a low capacity in using N-inputs to produce seeds. This capacity, commonly defined as N-Use Efficiency (NUE, Moll et al., 1982) is therefore generally low in oilseed rape. NUE is usually subdivided in plant's ability to absorb N from the soil (N-Uptake Efficiency, NUpE) and the ability to use the N absorbed by the plants to produce the seed yield (N-Utilization Efficiency, NUtE). NUtE includes the abilities of plants to assimilate N (N-assimilation efficiency) and to remobilize N from senescing to growing organs (N-remobilization efficiency) (Girondé et al., 2015).

Excessive N fertilization can lead to high N losses (Di and Cameron, 2002), giving rise to soil and water pollution by nitrate leaching and to air pollution by GHG emissions. In addition, the production of mineral N fertilizer is very demanding in terms of fossil-fuel energy (Robertson and Vitousek 2009), and N fertilizer is among major costs in oilseed rape production. Although progress has been made to optimize N-fertilization management, more than half of the worldwide N applied to crops is currently lost into the environment (Lassaletta et al., 2014). These losses are depending on the mineral N not removed from fields after harvest of the previous crop, and can lead, through runoff, to nitrate leaching, and/or the volatilization of nitrous oxide or ammonia (Billen et al., 2013). Nitrogen losses in the field can be reduced both by optimizing N-fertilization management (Keeney 1982) and by improving the NUE of the crop (Garnett et al., 2009). N-fertilization management involves more precise fertilizing practices, such are better coordination of N with soil water status and better matching of fertilizer requirements to soil properties (Garnett et al., 2009; Sieling and Kage, 2010; Simonin et al., 2017).

II) Scientific issues

II.1. A better understanding of the carbon and nitrogen functioning and its genotypic variability to improve oilseed rape yield under lower N-conditions.

Improving oilseed rape adaptation to lower N-conditions

Improving the oilseed rape adaptation to lower and fluctuating N availability, is one of the current challenges in breeding research to improve seed yield. N-efficient varieties should exhibit improved or maintained seed yields under lower-N conditions by efficiently capturing the N available in the soil throughout the crop cycle (higher NUpE) and/or by better assimilating, storing and remobilizing N to produce large quantities of seeds (higher NUtE) (Bouchet et al., 2016). However, oilseed rape NUE is a complex trait linked to plant growth, N availability, and C- and N-related processes over the entire crop cycle (Diepenbrock, 2000). One key lever to improve NUE relies on optimizing C and N resource acquisition and recycling them to the plant's reproductive parts (Ulas et al., 2013). Thus, improving oilseed rape NUE requires a thorough knowledge of the entire plant's functioning, especially under low N inputs, to analyze and dissect whole-plant complex dynamic traits related to development and growth and assess their genetic variability.

Complexity of the C and N fluxes

Processes impacting the C and N (C-N) fluxes remain relatively unexplored in oilseed rape (Song et al., 2020). The C-N functioning of a plant is complex because it emanates from the two major biogeochemical carbon and nitrogen cycles, which are themselves complex and interconnected. This complexity has been evidenced at the field level (Figure 0.4.A), but also in lower-level studies, either regarding the C-N biochemistry at the plant level (Figure 0.4.B), or regarding shoot-root coordination of metabolic processes involving hormones or transcription factors (Figure 0.4.C), or regarding the complex genes network (Figure 0.4.D). Moreover, C and N functioning is modulated by genotype-environment interactions, resulting either from natural genetic variation or from breeding. This leads to variable levels of phenotypic plasticity in the short and medium terms. Thus, to decipher plant adaptation to N availability, the interacting processes involved in C-N functioning, especially C-N acquisition and use, need to be considered at the whole plant scale by integrating source-sink dynamics (Xu et al., 2012) and crop architectural plasticity. Therefore, studying those whole plant processes using an ecophysiological approach seems to be promising.

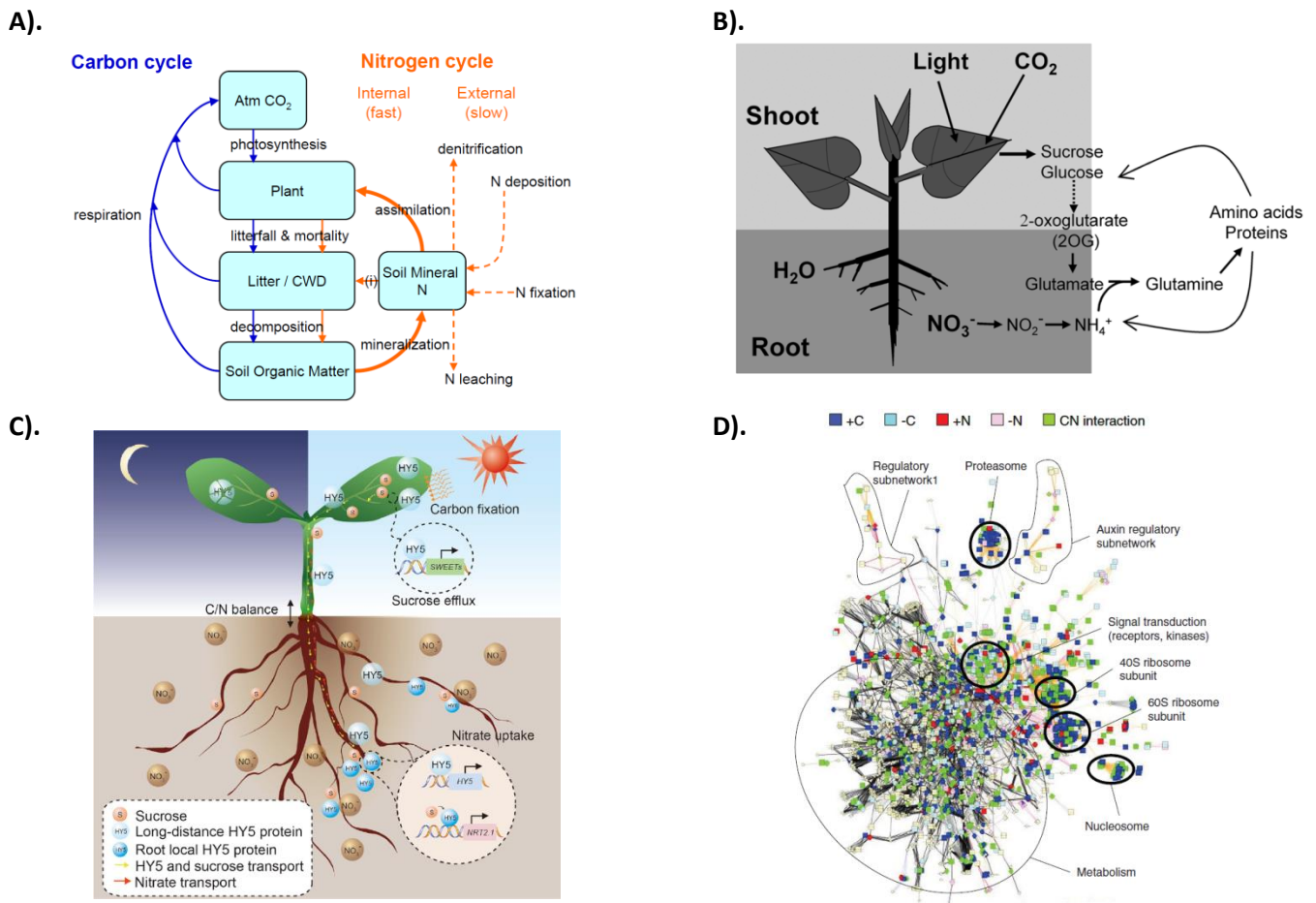


Figure 0.4. Multiscale complexity of plant's carbon and nitrogen functioning.

A. Biogeochemical carbon and nitrogen cycles and interacting feedbacks at the field level. Blue arrows depict C processes such as plant atmospheric C-uptake by photosynthesis; biomass losses from plant to litter and coarse woody debris (CWD); decomposition of plant biomass generating soil organic matter; respiration by both plants and heterotrophic organisms returning CO₂ to the atmosphere. Orange arrows depict N processes, differentiated between rapid internal cycling (solid arrows), and slower fluxes between land pools, the atmosphere, and ground water (dashed arrows). The thick orange arrow depicts soil organic matter decomposition, which releases both CO₂ to the atmosphere and N from the organic matter (mineralization). (From Thornton et al., 2009).

B. Carbon and nitrogen nutrient balance at the whole plant level depicting the tightly coordinated C and N metabolisms. 2-oxoglutarate is an important intermediate product of C metabolism serving as the C-skeleton for the synthesis of glutamate. The ammonium (NH₄⁺) resulted from primary N assimilation from nitrate (NO₃⁻) is then incorporated to glutamate, and glutamine is synthesized. Other amino acids are synthesized by using NH₄⁺ donated from glutamate and glutamine, and therefore essential proteins for the C and N metabolism can be synthesized. (From Zheng 2009).

C. Shoot-to-Root coordination processes of carbon and nitrogen acquisition involving hormones or transcription factors at the molecular level. The transcription factor HY5 is a shoot-to-root mobile signal that mediates light-responsive coupling of shoot growth and C assimilation with root growth and N uptake in *Arabidopsis thaliana*. HY5 mobility thus contributes to maintain a homeostatic balance between whole plant C and N metabolism in response to a fluctuating environment. (From Chen et al, 2016).

D. Arabidopsis thaliana subnetwork controlled by C, N or CN at the gene level. The different genes and functional associations between them were labeled and combined into a single network graph. Protein-coding genes, miRNAs, or metabolites are represented as nodes, and color and shapes have been assigned to differentiate them according to their function. (From Gutierrez et al., 2007).

Moreover, for a given environment, genotypes can exhibit several pathways, in terms of dynamics of resource uses, growth, and development to achieve a given biomass or seed production. For example, depending on the aerial architecture and phenology, the evolution of radiation interception will be different, with quantitative consequences on biomass production and allocation. Symmetrically, depending on the root system architecture, the capture of nitrogen resources might vary between genotypes. As a feedback, the modified absorption of N, as well as its variable allocation between organs, might modify the aerial architecture and consequently the radiation interception. The coordinated regulations and feedbacks make the oilseed rape plant very plastic to C-N variation in its environment, *e.g.* [Cong et al. \(2020\)](#). This plasticity shows the importance of studying the dynamics of C-N processes during the whole plant life to better understand the state of the plant at harvest.

To better understand this complex C-N functioning and its dynamics, a modeling approach has been used in several studies. Among the existing modeling approaches, structure-function approaches are preferred because the simultaneous consideration of development processes (which result in the plant structure) and functional processes that take place within a given structure is essential to address resource acquisition ([Fournier et al., 2007](#); [Pagès, et al., 2016](#)). However, building such models dedicated to the response of oilseed rape genotypes to nitrogen limitation are still running research actions ([Böttcher et al., 2020](#)).

Is the oilseed rape a relevant scientific model?

Oilseed rape, also called rapeseed, is a cultivated specie belonging to *Brassicaceae* family (formerly called *Cruciferae*), the same family as *Arabidopsis thaliana*, the most studied dicot plant model specie. *Arabidopsis thaliana*, has a broad geographical distribution and consequently is subject to varying nutritional environments, hence it has largely used as a model for studying possible contrasting adaptation to nutrient availability ([Loudet et al., 2003](#); [Richard-Molard et al., 2009](#); [Chardon et al., 2010](#); [Chevalier and Rossignol, 2011](#); [Krapp et al., 2011](#); [Pessemier et al., 2013](#); [Gruber et al., 2013](#)).

The phylogenetic proximity between *Arabidopsis thaliana* and oilseed rape might suggest, *i.e.* through synteny, a direct transposition of research on *Arabidopsis thaliana* to oilseed rape. However, compared to *Arabidopsis thaliana*, the *Brassica napus* genome is very complex. It originated from a recent combination of two distinct genomes (AA and CC), themselves originated from a triplication of the ancestral brassica genome ([Chalhoub et al., 2014](#)). As this “Brassica triplication” event did not occur during the evolution of *Arabidopsis thaliana*, the transposition of *Arabidopsis thaliana* results to oilseed rape remains very challenging ([Song et al., 2020](#)). This synteny has been successfully used for improving “simple” agronomic traits such as pod shatter ([Stephenson et al., 2019](#)).

However, regarding plant's development and carbon and nitrogen functioning, oilseed rape has a more complex root system compared to *Arabidopsis thaliana*, with a taproot and fine roots, as well as very plastic shoot architecture. Moreover, oilseed rape exhibits leaves' losses since early growing stages, which might impact de C-N fluxes in the plant and N mineralization in the soil. These specificities of the oilseed rape plant make relevant research on its functioning and on its specific genetic variability.

II.2. Phenotyping oilseed rape response to N-availability

Characterizing oilseed rape responses to N-availability requires high-throughput accurate phenotyping of various plant traits and adapted experimental designs and devices for managing plants along with a correct estimation of plant N status and soil N-availability, especially under low-N nutrition (Han et al., 2015). Indeed, the scope of improvement for NUE has been limited due to the inherent difficulty of phenotyping the whole plant system, including roots (Garnett et al., 2009; Postma et al., 2014). The development of high-throughput phenotyping facilities, especially for the root system of crop plants is currently under progress. The development of such facilities raises several scientific questions leading to an emerging discipline, the plant phenomics e.g. Tardieu et al. (2017) or Ninomiya et al. (2019), whose results would be of high interest for phenotyping the response of oilseed rape to nitrogen fertilization.

Moreover, NUE is usually evaluated in the field at harvest. However, identifying traits for phenotyping NUE at early growing stages can accelerate breeding processes by screening large numbers of genotypes and environments under controlled growing conditions and avoiding waiting for seed maturity. However, high-throughput phenotyping facilities are not adapted for growing oilseed rape until seed maturity without limiting plant growth and development. Nevertheless, the relevance of assessing NUE before seed maturity is still unclear and should be further investigated (Balint and Rengel 2008; Bouchet et al., 2016). Indeed, a model-assisted phenotyping approach might be relevant. Such approaches allow considering plants' functioning in interaction with their environment, assessing the compensation between dynamic processes by considering the genetic variation of parameters rather than variables, which may be useful in oilseed, characterized by very high plasticity.

III). From societal and scientific issues to a doctoral research work

To respond to the societal issues regarding oilseed rape and its N fertilization, an agronomic engineering approach may be chosen to optimize soil and crop management practices. However, as underlined in the previous section, the complexity of the carbon-nitrogen (C-N) functioning of this crop, the high number of *a priori* related traits, and the associated genetic variability first require more knowledge and understanding of the dynamic response of oilseed rape to low N, which constitutes a challenging research topic for Ph.D. work on plant ecophysiology.

This Ph.D. work has focused on obtaining a better understanding of the ecophysiological processes determining NUE enhancement in winter oilseed rape by identifying, hierarchizing, and evaluating functional traits that support the observed genotypic variation during the crop cycle under low-N conditions, with a particular emphasis on above-ground traits.

In Chapter I, we present the identified research objective of this Ph.D. work, as well as the research strategy chosen to reach this objective. In Chapter II, we present a literature review, conducted to identify first the gaps of knowledge and the relevant questions to address the importance of N availability for oilseed rape development and growth, and second the impact of low-N availability on plant functioning and yield elaboration over the whole crop cycle. In Chapter III and Chapter IV, we present the experimental and model-based approaches respectively, carried out to identify the main contributive processes and plant traits involved in the tolerance of rapeseed to low-N availability, in the form of two scientific papers. The discussion of these results and the perspectives drawn from them are presented in Chapter V.

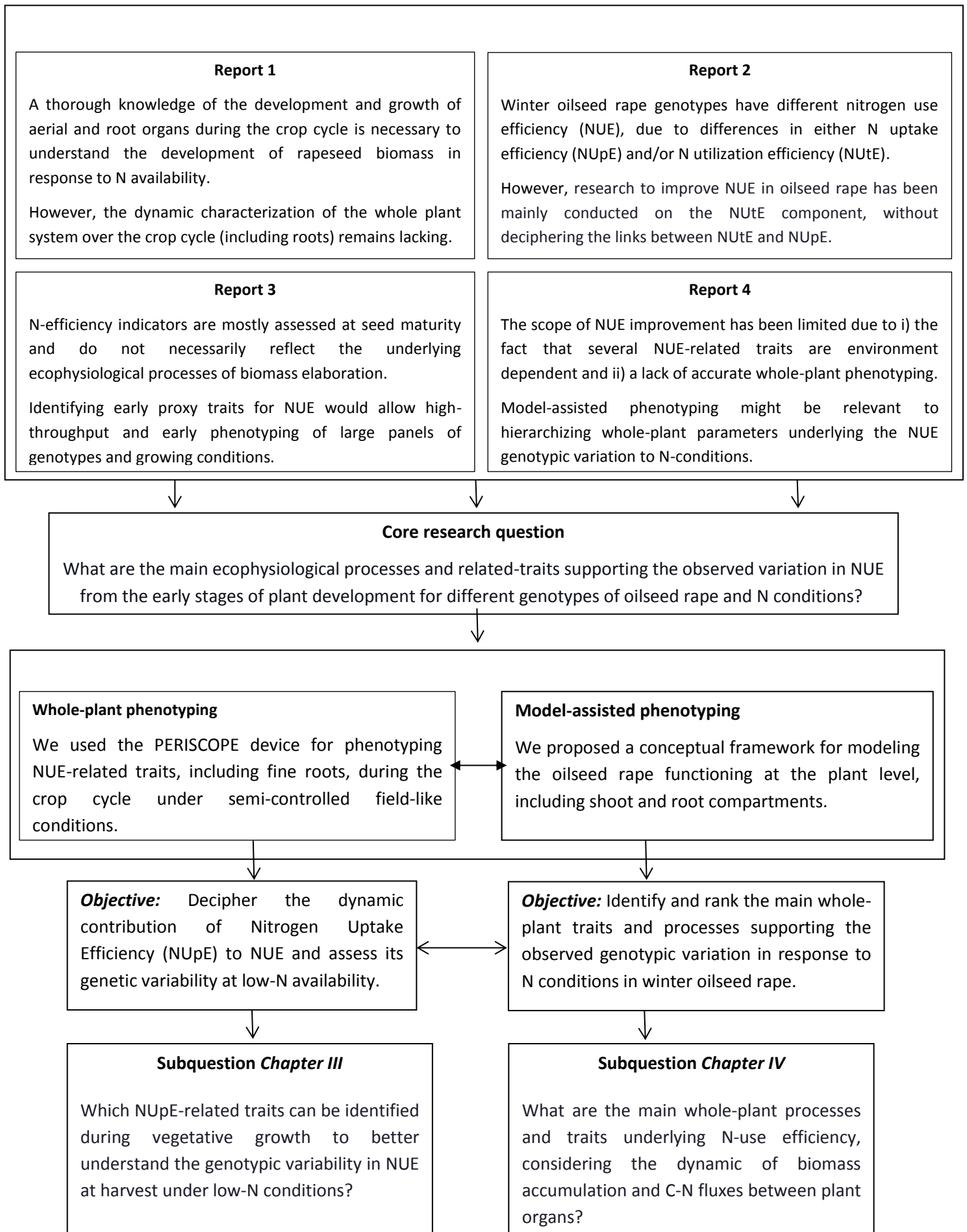


Figure I.1. Structure of the PhD work and research questions

CHAPTER I. PRESENTATION OF THE Ph.D. THESIS

1.1. From research issues to a Ph.D. objective

There is a compelling need to identify relevant traits for breeding efficient oilseed rape genotypes under lower N conditions. As seen in the previous section, the oilseed rape responses to soil N availability result from a complex set of interacting processes throughout the plant cycle and displaying wide genetic and environmental variability. Thus, identifying relevant traits for breeding requires a better understanding of a plant's functioning from the canopy to organ level in response to low-N availability. The multiple processes and traits linked to the plant response to low-N availability require prioritizing and focusing the research actions for deciphering the underlying process driving biomass elaboration. Therefore, we started this Ph.D. study by an in-depth review of the scientific literature (presented in Chapter II). This review led us to highlight four main points (Figure I.1):

- First, the concept of nitrogen use efficiency (NUE), although varying among communities and authors, appears to be of central interest for such an issue. Accordingly, NUE has been identified as a target by breeders, and few oilseed rape varieties have been developed in this sense. Indeed, thorough knowledge of aerial and root organ development and growth during the crop cycle is still required to understand oilseed rape biomass elaboration in response to N inputs.

- Second, plant N uptake is an important process for NUE. One lever to improve NUE might therefore rely on the optimization of this process, particularly under low N conditions. Specifically, the optimization could deal with N uptake efficiency during vegetative growth—which leads to the constitution to the N-available pool recycled to the reproductive parts—and with its duration with a prolonged N uptake from flowering onwards. To achieve that, the root system characteristics (*i.e.* morphology) and physiological processes (*i.e.* N absorption and growth) and their genotypic variation should be further studied and therefore efficiently and dynamically measured.

- Third, N efficiency indicators, such as NUE, are mostly assessed at seed maturity, which does not necessarily reflect the dynamic of the underlying ecophysiological processes of biomass elaboration and partitioning between organs. Identifying early proxy traits for NUE would allow a dynamic characterization of NUE and early phenotyping of larger panels of genotypes and growing conditions, hopefully at high-throughput.

- Fourth, to hierarchize the main whole-plant traits driving biomass elaboration during the growing cycle under low-N conditions, and therefore underling NUE, a model-assisted phenotyping approach might be relevant. Such an approach has been successfully used in other crops to analyze the genotypic variability of whole-plant traits associated with N uptake and in response to N conditions. However, until very recently (Böttcher *et al.*, 2020), no whole-plant ecophysiological model had been developed linking the shoot and root systems in oilseed rape in order to explain the dynamics of C and N resource acquisition and allocation at the crop scale. To our knowledge, no such model exists neither at the plant scale in oilseed rape.

We therefore focused this doctoral work on the following **research question**:

What are the main ecophysiological processes and related traits supporting the observed variation in NUE from the early stages of plant development for different genotypes of oilseed rape and N conditions?

The literature review presented in Chapter II led us to focus on *a priori* relevant processes and traits, and therefore to decompose the research objective into **the two following sub-questions**:

- (i) Which NUpE-related traits can be identified during vegetative growth to better understand the genotypic variability in NUE at harvest under low-N conditions?
- (ii) What are the main whole-plant processes and traits underlying N-use efficiency, considering the dynamic of biomass accumulation and C-N fluxes between plant organs?

To address these questions, we adopted a **research strategy** combining an experimental approach to decipher the dynamic response of winter rapeseed genotypes to low-N conditions, with particular attention to the root system (Chapter III), and a model-assisted phenotyping approach to hierarchize the main whole-plant processes considering the dynamics of C and N fluxes between whole-plant organs to identify promising traits (Chapter IV). The structure of the thesis work is summarized in Figure I.1.

In the following section, we will detail our research strategy and the resulting experimental design.

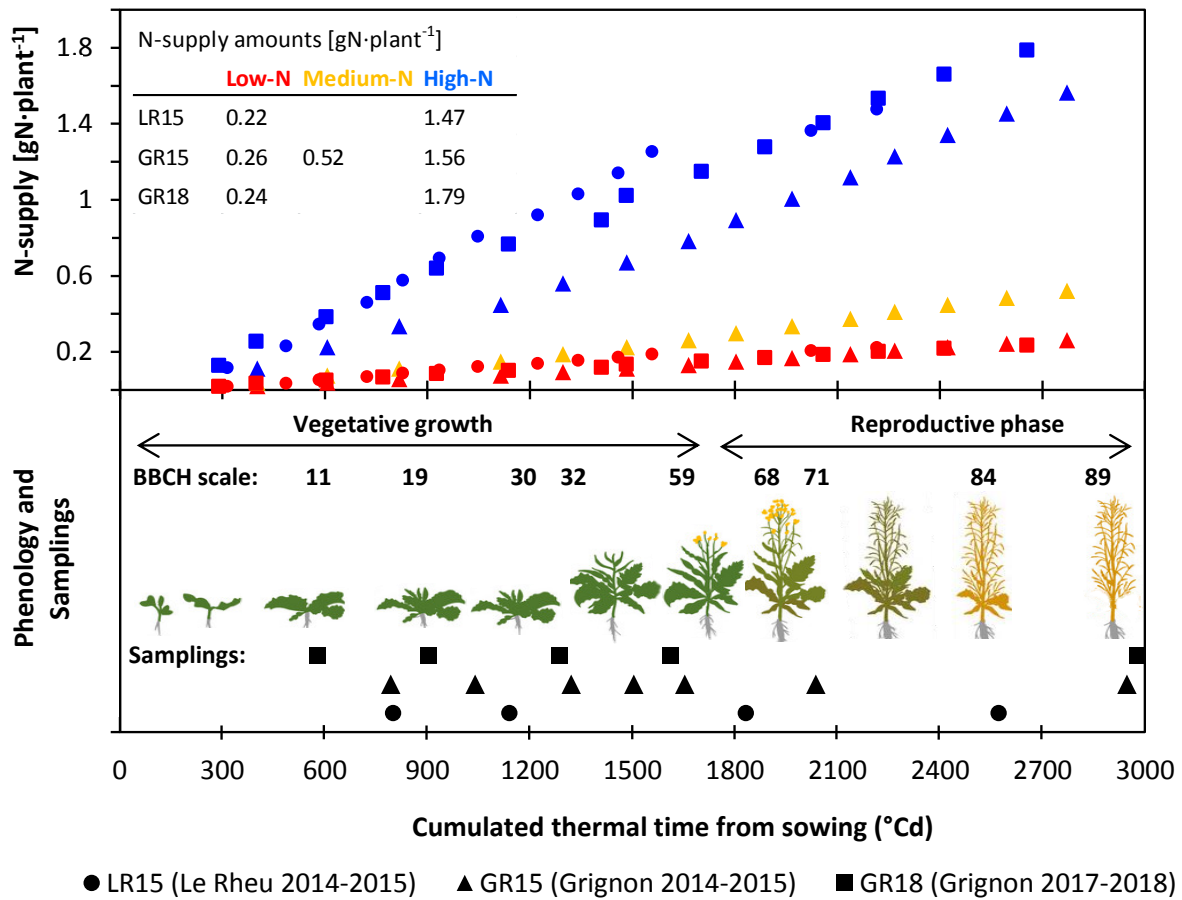


Figure I.2. Overview of the three experiments (LR15, GR15, and GR18) in terms of N supplies and sampling dates related to plant phenology. The upper plot corresponds to N management and represents the N-supply dynamics during the crop cycle (expressed in growing degree days cumulated from sowing). The included table presents the total amounts of N supplied at the end of the plant cycle. The lower plot corresponds to the phenology of winter oilseed rape and the intermediate sampling points of each experiment. Thermal time is expressed in growing degree-days from sowing using a base temperature of 0°C. The main growing stages are indicated according to the BBCH scale: rosette autumnal growth (BBCH 11–19), beginning of stem elongation after overwintering (BBCH 30–32), end of vegetative growth before flowering (BBCH 59), end of flowering and beginning of pod development (BBCH 68–71), and seed maturity (BBCH 84–89). Symbols correspond to experimental sites and climatic years (circles for LR15, triangles for GR15, and squares for GR18). Colors indicate the N-supply condition (blue, non-N-limiting; yellow, medium-N; and red, N-limiting).

1.2. Research strategy and dedicated experimental setup

1.2.1. Research strategy

We chose an approach combining experimental field-like phenotyping and model-assisted phenotyping. N-use efficiency is usually measured as a crop balance at harvest: specifically, the ratio of seed yield to soil N available. The effects of low-N availability are quantified at the canopy scale at maturity, but a better understanding of the underlying NUE processes at the plant scale, as well as their modulation by N deficiency, is needed. Furthermore, considering the high plasticity of oilseed rape development in response to the climatic conditions, and compensatory capacities of the organs between the vegetative and the reproductive phase, the plant-scale processes deserve to be studied under field conditions or, at least, under field-like reconstructed canopies throughout the crop growth cycle. We therefore choose an adapted culture device called PERISCOPE, (Bissuel-Belaygue et al., 2015) ensuring field-like conditions in terms of shoot interactions between plants and root-explored soil volume, and procuring access to the individual root system of each plant from sowing to harvest. In our experiments, the plant response to N availability was investigated by evaluating NUE-related functional traits over the crop cycle (*i.e.* total shoot and root dry matter and C and N content) and integrative traits such as seed yield and NUE, assessed at the end of the crop cycle. Several intermediate destructive measurements allowed us to accurately characterize NUE dynamics, assessing how the individual developmental processes led to the final seed yield and NUE at harvest.

For the model-assisted phenotyping approach, we proposed a conceptual framework describing winter oilseed rape C and N functioning, including all shoot and root compartments. It was designed from the model called ARNICA developed for *Arabidopsis thaliana*, (Richard-Molard et al., 2009) (Chapter II. Figure II.11). The proposed conceptual framework was then used for the trait analysis, as it offers an explicit and dynamic description of whole-plant growth, allowing us to identify and hierarchize the main processes supporting the observed genotypic variation of biomass elaboration in response to N conditions, and therefore of NUE if considering the ratio of produced plant biomass to N-available. All the model state variables were chosen to be measurable on the plants grown on the PERISCOPE device. Modeling was only used here as a framework for analyzing and prioritizing traits likely to be phenotyped.

However, measuring those traits required destructive measurements throughout the crop cycle, including time-consuming measurements of root traits. Hence, depending on the experiment, we acquired those variables on a limited number of genotypes across different N supplies. We investigated a total of seven genotypes of winter oilseed rape (*Brassica napus* L.) in three different experiments, combining a maximum of three contrasting N supplies. The genotypes were chosen to represent winter oilseed rape diversity as they were released between 1980 and 2004 and represent ancient ‘++’ (high glucosinolates and high erucic acid contents) or modern ‘00’ types (low glucosinolates and low erucic acid). The genotypes were chosen from a large panel, previously phenotyped in the field, for their contrasting seed yield responses to N input levels (Bouchet et al., 2016).

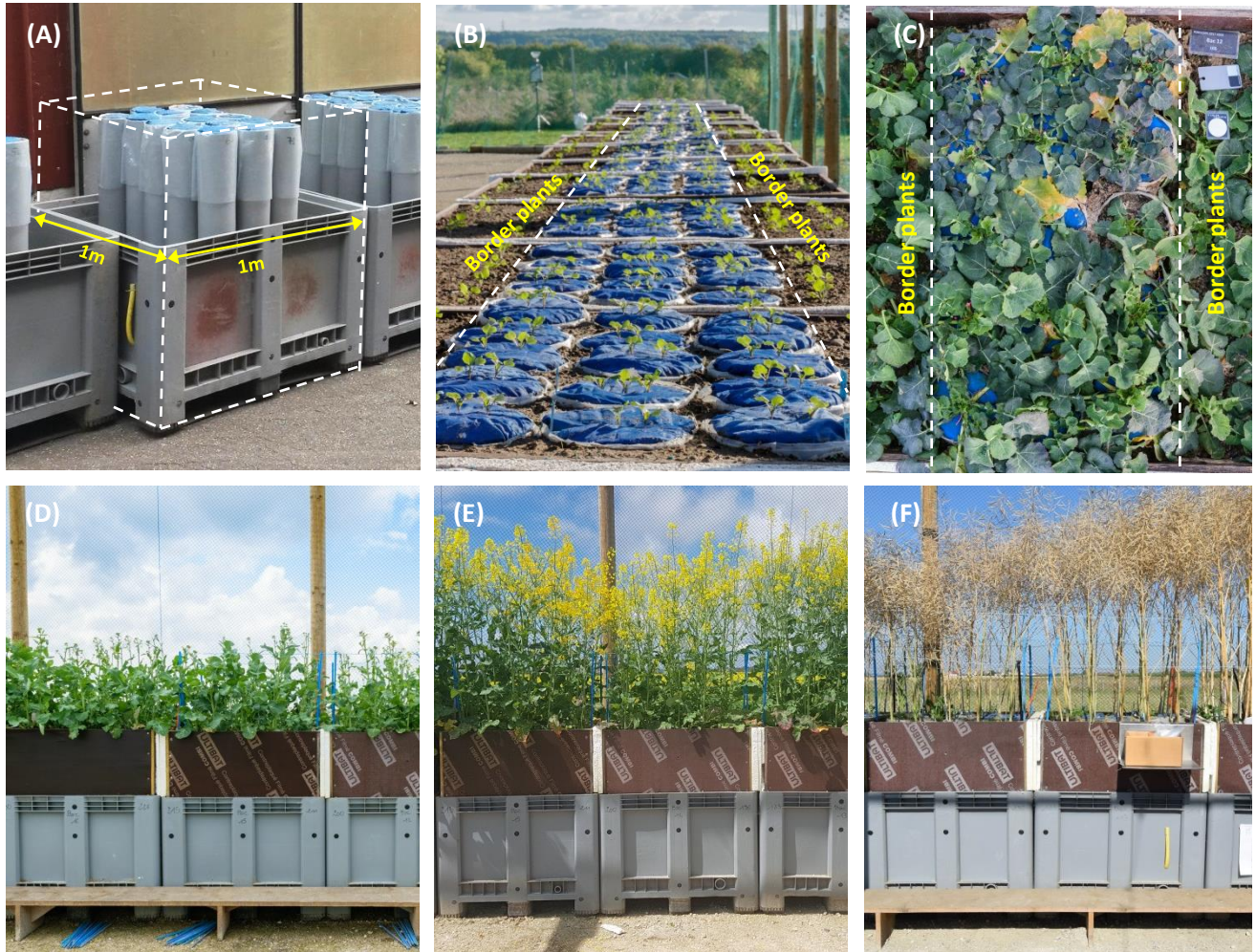


Figure 1.3. Experimental device used for phenotyping N-use-efficiency-related traits during the whole crop cycle. Winter oilseed rape plants were grown under field-like conditions in a reconstructed canopy system (PERISCOPE device) allowing individual root and shoot measurements. Plants were grown in individual tubes 1 m high and 0.16 m in diameter, grouped into containers of 1 m³, placed outside and therefore submitted to field climate (A). Each tube was filled with substrate and regularly supplied with nutrient solution. In containers, the space between tubes was filled with soil to ensure the thermal insulation of root parts (B). Two rows of border plants were sown around the tubes to mimic bioclimatic field conditions. Six seeds were sown in each tube (B). After thinning, a single plant was kept per column, leading to a homogeneous canopy of 35 plants/m² (C, aerial view of one container), which were grown for the whole crop cycle until harvest (D, vegetative growth; E, flowering; F, seed filling and ripening). Photos correspond to the experiment conducted in Grignon in the 2017–2018 cropping season (GR18).

1.2.2. Experimental setup

Three experiments were conducted at two different sites and over two climatic years, in Le Rheu from 2014–2015 and in Grignon from 2014–2015 and 2017–2018, hereafter referred to as LR15, GR15, and GR18, respectively. The LR15 and GR15 experiments were conducted before I began my Ph.D., whereas I personally designed and conducted the GR18 experiment. Experiments were conducted using the PERISCOPE device (Bissuel-Belaygue et al., 2015). An overview of the experiments and a description of their specific characteristics and modalities similarities are presented in Figure I.2. More detailed descriptions of the growing conditions and trait measurements are given in Chapter III and Chapter IV. The experiments performed are complementary in terms of genotypes tested and sampling stages (Figure I.2), and used a common methodology for data measurements. We were careful to compare genotypes with contrasting seed yield in the field but similar growth-cycle durations and dates of flowering (no more than 8 days between the two extreme genotypes) to minimize confounding effects between phenology and NUE processes. The genotype AVISO was tested as a control in all experiments.

Plants were grown in tubes (Figure I.3) and grouped into containers of 1 m³ to obtain a reconstructed canopy with a density of 35 plants m⁻², commonly used in Europe (Terres Inovia, 2019). In containers, the space between tubes was filled with soil to ensure the thermal insulation of the root system. In addition, to avoid edge effects, two rows of plants were sown on each edge of the container. We sowed six seeds in each column: after thinning, only one medium-sized plant remained in each tube. We harvested plants at multiple growing stages throughout the crop cycle. Sampling dates depended on the experiment but altogether result in a comprehensive dataset on the growing dynamics (Figure I.2). To simulate constant low- and high-N conditions during the crop-growth cycle, a mineral solution was supplied every 200 growing degree days from emergence to seed maturity, resulting in 13 to 14 applications during the growth cycle. Moreover, we maintained the soil moisture above 85% of field capacity, thus avoiding other uncontrolled stress (*i.e.* water stress and nutrient loss through leaching).

At each sampling date, we divided harvested plants into fractions: taproots, fine roots, leaves (green, senescing, and fallen), main stem, branch stems, and pods (including immature seeds or, when dehiscent, seeds and pod walls). Shoot traits (*i.e.* stem diameter, plant height, leaf number, number of branches, and pod and seed number) and root traits (*i.e.* secondary root number and tap root depth and length) were measured manually (Figure I.4). Dry matter and C and N content of the different plant fractions were measured, and the green leaf area and pod area were assessed. Soil horizons were sampled for N-mineral characterization and soil moisture quantification. To characterize the crop N nutritional status, we used the nitrogen nutrition index (NNI), calculated using the equation developed by Lemaire and Gastal (1997) (Chapter II, Figure II.1). The NNI was measured on three key phenological stages, common to all experiments: at the beginning of rosette growth in autumn (BBCH 16-19), during stem elongation in spring (BBCH 30-32), and at the end of inflorescence emergence, immediately before flowering (BBCH 59). The characteristics of the N conditions generated, including mean values of N supplies, mineral N initially present in the substrate, and NNI, are presented in Chapter IV (Table IV.2).

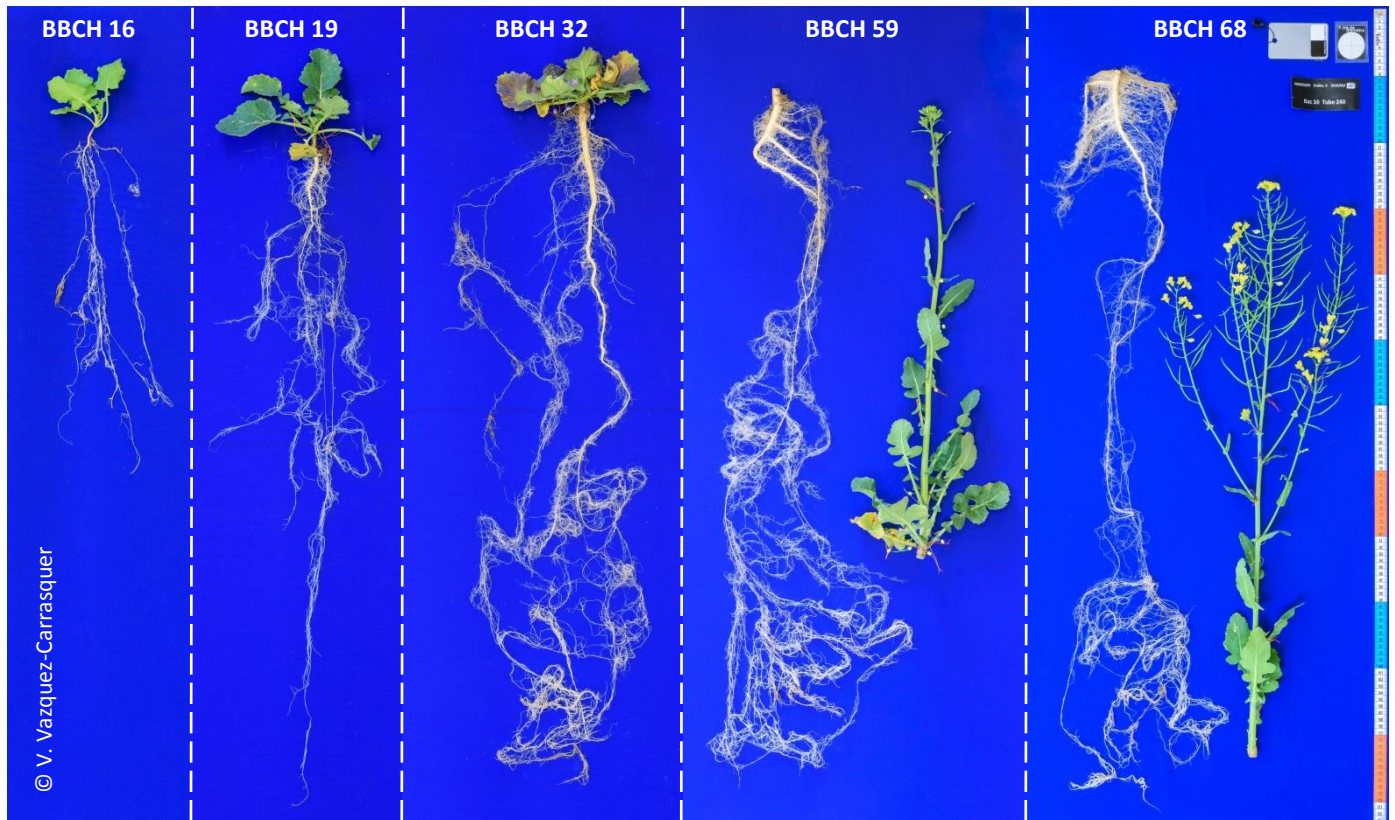


Figure I.4. Winter oilseed rape plants at five sampling stages during the crop cycle.

Using the PERISCOPE device, all individual plant fractions were collected, including fine roots and fallen leaves of each plant, until harvest. Five key growing stages are presented: BBCH 16–19, autumnal growth; BBCH 32, beginning of stem elongation after overwintering; BBCH 59, end of the vegetative growth immediately before flowering; and BBCH 68, end of flowering and beginning of pod development. Photos correspond to the cv. AVISO growing under low-N conditions in the experiment conducted in Grignon in the 2017–2018 cropping season (GR18). Scale colored bands correspond to 10 cm.

The methodological specificities and main objectives of each experiment are described below.

1.2.2.1. LR15 experiment (6 genotypes x 2 N supplies): This experiment was intended to characterize the genotypic variation in six winter oilseed rape growth and NUE at four key stages in response to two contrasting N conditions. The experiment was conducted at the INRA research station in Le Rheu (LR), located in Brittany, France (latitude 48°06'29.0"N; longitude 1°47'37.3"W), during the 2014–2015 cropping season. Six contrasting genotypes winter oilseed rape genotypes (**cv. AMBER, ASTRID, AVISO, EXPRESS, MOHICAN, and MONTEGO**) for seed yield, biomass accumulation, and amount of N absorbed in field conditions, were cultivated under two N supplies (N-limiting: equivalent to 25 kg N ha⁻¹; non-N-limiting: equivalent to 165 kg N ha⁻¹). Genotypes were experimented using a split-plot design allowing **five repetitions** (genotype x N supply). Each tube was filled with a soil-sand mixed substrate and was regularly supplied with nitrogen-free Hoagland nutrient solution to prevent water and other mineral stresses, except N. Nitrogen solution was supplied 13 times over the cycle, at intervals of approximately 200 growing degree days. Plants were harvested on **four sampling dates during the crop cycle**, including three intermediate samplings at the growing stages—BBCH 18 (autumnal growth), BBCH 31 (beginning of stem elongation), and BBCH 68 (end of flowering and beginning of pod development)—and a final sampling at seed maturity (Figure 1.2). These are key development stages determining changes in whole-plant C-N balance, as reported in the literature.

1.2.2.2. GR15 experiment (1 genotype x 3 N supplies): This experiment was intended to characterize in detail the dynamic growth of a single genotype (**cv. AVISO**) in response to three contrasting N conditions (equivalent to 29, 58, and 175 kg N ha⁻¹). The experiment was conducted at the INRA research station in Thiverval-Grignon (GR), France (latitude 48°50'21.7"N; longitude 1°56'48.4"E) during the 2014–2015 cropping season. Nitrogen solution was supplied 14 times over the cycle. Each column was filled using a mixture of attapulgitite and clay pebbles and regularly supplied with Hoagland solution. Plants were experimented using a complete block design allowing **six repetitions**. Plants were harvested on **seven sampling dates during the crop cycle**, including five samplings during the vegetative growth (BBCH 19, 20, 21, 30, and 59), one sampling at the end of flowering (BBCH 71), and one sampling at seed maturity.

1.2.2.3. GR18 experiment (5 genotypes x 1 N supply + 1 genotype x 2 N supplies): This experiment was intended to accurately characterize shoot and root growth throughout the vegetative phase of five oilseed rape genotypes (**cv. AMBER, AVISO, EXPRESS, MOHICAN, and OLESKI**) growing under low N supply. The experiment was conducted at the INRA research station in Thiverval-Grignon during the 2017–2018 cropping season. Plants were grown under a single N limiting supply (equivalent to 29 kg N ha⁻¹), except for AVISO, which was additionally cultivated under a non-limiting N supply (equivalent to 200 kg N ha⁻¹). Nitrogen solution was supplied 14 times over the cycle. Each column was filled using the same substrate than in GR15 and regularly supplied with Hoagland solution. Plants were experimented using an incomplete block design allowing **seven to eight repetitions** per genotype. Plants were harvested on **four sampling dates during vegetative growth** (BBCH 16, 19, 32, and 59), and one additional sampling was performed at harvest to quantify seed yield. In addition to the other measured plant traits described above for other experiments, the leaf area of the fallen leaves was measured.

CHAPTER II. BIBLIOGRAPHIC REVIEW

This chapter reviews previous literature investigating oilseed rape responses to low nitrogen availability. It aims to identify the main ecophysiological processes and related traits involved in these responses throughout the crop cycle, as well as their genetic variability. The concept of the Nitrogen Use Efficiency (NUE) is central for our study, but should be clarified with regard to the diversity of this term in the literature.

This chapter is organized as follows. First, the current definitions of NUE that can be used at the crop and plant level will be synthesized. Indeed, different ways of decomposing NUE will be presented in order to better understand the process related to NUE in winter oilseed rape. Then, the impact of nitrogen limitation on biomass elaboration during the oilseed rape growing cycle will be documented at canopy and plant level, as well as the impact on nitrogen components and whole plant traits. Genetic variation for N-associated traits and processes and current knowledge on genotype x nitrogen interactions will be examined. Finally, existing devices and approaches for the phenotyping of whole plant N-associated traits in oilseed rape will be described.

Table II.1: Definitions of nitrogen use parameters and occurrence for oilseed rape.

Eq.	Term	Formula and units	Definition	Defined; reviewed by:	Used in oilseed rape by:
(1)	Agronomic or Assimilation Efficiency (AE)	$AE = \frac{(SY_2 - SY_0)}{N_s} [kg\ kgN^{-1}]$	SY: Seed Yield in a plot with N-supplied fertilizer (SY ₂) and without N-supplied fertilizer (SY ₀). N _s : amount of N-supplied fertilizer. AE= RE x PE (Eq. 2 x Eq. 3)	[6]; [4]; [7]; [11]; [22]; [34]	[29]
(2)	Recovery Efficiency (RE)	$RE = \frac{(NUp_2 - NUp_0)}{N_s} x100 [\%]$	NUp: N-Uptake in aboveground biomass in a plot with N-supplied (NUp ₂) and without N-supplied (NUp ₀). N _s : amount of N-supplied fertilizer.	[4]; [6]; [7]; [11]; [22]; [34]	[10]; [29]; [35]
(3)	Physiological Efficiency (PE)	$PE = \frac{(SY_2 - SY_0)}{(NUp_2 - NUp_0)} [kg\ kgN^{-1}]$	SY: Seed Yield in a plot with N-supplied fertilizer (SY ₂) and without N-supplied fertilizer (SY ₀). NUp: N-Uptake in aboveground biomass at maturity in a plot with N-applied (NUp ₂) and without N-applied (NUp ₀).	[4]; [6]; [7]; [11]; [22]	[8]; [9]; [18]; [33]; [36]
(4)	N-Use Efficiency or partial Factor of Productivity (NUE)	$NUE = \frac{SY\ or\ PlantDM}{N_s} [kg\ kgN^{-1}]$	SY: Seed Yield in a given N-condition. Plant DM: aboveground Dry Matter. N _s : amount of N-supplied fertilizer. NUE= NUpE x NUtE (Eq. 5 x Eq. 6)	[3]; [7]; [11]; [12]; [14]; [20]; [22]; [23]; [32]	[2]; [9]; [15]; [16]; [17]; [21]; [26]; [27]; [28]; [37]
(5)	Physiological N-Utilization Efficiency (NUtE)	$NUtE = \frac{SY\ or\ PlantDM}{NUp} [kg\ kgN^{-1}]$	SY: Seed Yield in a given N-condition. Plant DM: aboveground Dry Matter. NUp: N-Uptake in aboveground biomass in a given N-condition.	[3]; [11]; [12]; [14]; [20]; [22]; [23]; [32]; [34]	[8]; [10]; [13]; [15]; [16]; [17]; [21]; [26]; [28]; [30]; [33]; [37]
(6)	N-uptake efficiency (NUpE)	$NUpE = \frac{NUp}{N_s} [kgN\ kgN^{-1}]$	NUp: N-Uptake in aboveground biomass. N _s : amount of N-supplied fertilizer	[3]; [11]; [12]; [14]; [20]; [22]; [32]; [34]	[2]; [15]; [21]; [26]; [27]
(7)	Crop recovery efficiency of N applied or fertilizer efficiency (FE)	$FE = \frac{(NUp_2 - NUp_0)}{(N_s)} [kgN\ kgN^{-1}]$	NUp: N-Uptake in aboveground biomass in a plot with N-supplied fertilizer (NUp ₂) and without N-supplied fertilizer (NUp ₀). N _s : amount of N-supplied fertilizer at a given condition (N _{s2}).	[8]	[8]; [9]
(8)	Utilization index (UI)	$UI = PlantDMx \frac{PlantDM}{NUp} [g\ gN^{-1}]$	Plant DM: aboveground Dry Matter. NUp: N-Uptake in aboveground biomass in a given N-condition.	[12]; [24]	
(9)	N-harvest index (NHI)	$NHI = \frac{N_{SY}}{NUp} x100 [\%]$	N _{SY} : N-quantity in seeds. NUp: N-Uptake in aboveground biomass in a given N-condition.	[1]; [5]; [32]; [34]	[9]; [13]; [15]; [16]; [17]; [19]; [21]; [25]; [26]; [30]; [31]; [33]

References: [1] Austin et al. (1977); [2] Berry et al. (2010); [3] Bouchet et al. (2016); [4] Cassman et al. (2002); [5] Cormier et al. (2016); [6] Craswell and Godwin (1984); [7] Dobermann (2005); [8] Dresbøll et al. (2014); [9] Dresbøll et al. (2016); [10] Girondé et al. (2015); [11] Good et al. (2004); [12] Han et al. (2015); [13] He et al. (2017); [14] Hirel et al. (2007); [15] Kessel et al. (2012); [16] Koeslin-Findeklee et al. (2014); [17] Koeslin-Findeklee et al. (2016); [18] Li et al. (2016); [19] Malagoli et al. (2005); [20] Moll et al. (1982); [21] Nyikako et al. (2014); [22] Rathke and Behrens (2006); [23] Sattelmacher et al. (1994); [24] Siddiqi et al. (1981); [25] Sorten et al. (2003); [26] Stahl et al. (2015); [27] Stahl et al. (2017); [28] Stahl et al. (2019); [29] Su et al. (2014); [30] Svečnjak and Regel (2006); [31] Ulas et al. (2013); [32] Van Bueren and Struik (2017); [33] Wang et al. (2016); [34] Xu et al. (2012); [35] Yousaf et al. (2016); [36] Barlóg et al 2004); [37] Schulte auf'm Erley (2011).

2.1. Several definitions exist for N use efficiencies and related parameters

N-use efficiency (NUE) has been defined in several ways depending on the purpose (agronomic, physiological or economic), the level of study (canopy or plant level) and the yield parts of the crop (biomass or seed yield), as reviewed by [Good et al. \(2004\)](#), [Doberman \(2005\)](#), [Xu et al. \(2012\)](#) and [Han et al. \(2015\)](#). In addition, NUE definition also differ according to the growing stage of the plant (at seed maturity or at earlier stages) and to the estimation of the soil N availability for the plants (N-supplied by fertilization only or total mineral N-available in the soil). From a physiological point of view, it is also important to distinguish NUE defined as a process from NUE defined as a breeding phenotyping target ([Cormier et al., 2016](#)). The different definitions and related parameters relative to N-use efficiency are presented below, with the details and the equations summarized in Table I.1.

The agronomic efficiency, also called assimilation efficiency (Eq. 1) ([Craswell et al., 1984](#)), measures how efficiently the fertilizer is converted into seed yield at the canopy level. It is expressed as the product of the efficiency of N recovery from N-supplied (Recovery efficiency, Eq. 2) by the efficiency with which the plant uses each additional unit of N-uptake (Physiological efficiency, Eq. 3). Similarly, for [Moll et al. \(1982\)](#), N-use Efficiency represents the canopy capacity to transform N-input into seed yield (Eq. 4), and was defined as the product of two components: i) the ability of crop to capture N from the fertilization or available in the soil (Nitrogen Uptake Efficiency, NUpE; Eq. 6) and ii) the plant ability to use the N absorbed to produce seeds (Nitrogen Utilization Efficiency, NUtE; Eq. 5). At the plant level, other authors decompose NUtE between the abilities to assimilate (N assimilation efficiency; NAE) and to remobilize N from senescing or sink organs to growing organs (N remobilization efficiency; NRE) (reviewed by [Masclaux-Daubresse et al., 2010](#)). [Dresbøll et al. \(2016\)](#), consider N-uptake fertilizer efficiency (Eq. 7) as the difference between the amount of N uptaken in high *versus* low N-conditions per unit of N-supplied, thus indicating the ability of the plant/canopy to capture new nitrogen resource when available in the soil. Other definitions include the N-utilization index (Eq. 8), which factors in the absolute amount of above-ground biomass produced per unit of N-absorbed ([Siddiqi et al., 1981](#)). Finally, Nitrogen Harvest Index (NHI, Eq. 9) is defined as the ratio of N amount in the seeds to the total N amount uptaken in above-ground plant biomass at harvest time, thus representing the efficiency of N translocation to the seeds. NHI is analogous to HI (Harvest Index), which is the ratio of seed to above-ground plant biomass.

In addition to the multiple definitions of NUE, the assessment of the N-efficiency variables has been conducted in various sites, climatic years, genotypes, and N-conditions. Indeed, when discussing improvements of NUE or comparing results provided by different studies, it is necessary to take into account how NUE is calculated as well as the conditions under which it has been quantified [Cormier et al., \(2016\)](#) pointed up two main issues: i) the quantification of the total amount of N in the plant and ii) the estimation of N-available in soil for the crop during cycle.

The total amount of N in the plant has been usually estimated by measuring the aerial parts, excluding roots. Not taking into account roots' N-quantity overestimates NUtE and underestimates NUpE.

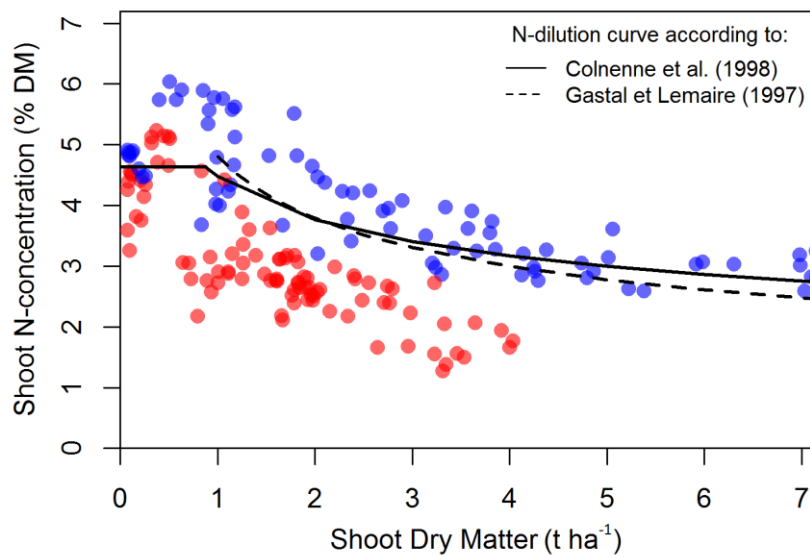


Figure II.1 Critical N dilution curves. The critical N dilution curve established by Colnenne et al. (1998) for winter oilseed rape ($N=4.48 \times DM^{-0.25}$) is represented by a solid line; the generic curve established by Gastal and Lemaire (1997) for C3 crops ($N=4.8 \times DM^{-0.34}$) is represented by a dashed line. Points correspond to measurements of Aviso individual plants growing under limiting (red) and non-limiting (blue) N-conditions in the LR15, GR15 and GR18 experiments (cf. Chapter I, 1.2.2 Experimental set up).

Soil N-available for the crop is difficult to estimate, as it results from a balance between the N-inputs of the system, which derive from residual N in the soil at sowing, aerial N deposition, net mineralization of organic matter and applied N-fertilization, and the N-outputs such as N leaching losses, N-volatilization, and N-uptake by the crop. Nevertheless, a large majority of studies reported NUE and its components based on N-input from fertilizer, without considering other N sources such as mineral N present at sowing in the soil and N mineralized from the organic matter during the plant cycle. This reduces the estimation of total soil N-available for the plants and increases NUpE. In the field, the mineral N-availability highly fluctuates along the crop cycle due to environmental factors, such as water deficit in the soil (Recous et al., 2015; Machet et al., 2017) or water-logging (Rathke et al., 2006). Differences in the estimation of the N-available are even larger when comparing field experiments to pots experiments (ie. soil volume, N-leaching and water balance) (Poorter et al., 2012, Hohmann et al., 2016 and He et al., 2017). Comparing results across different studies becomes therefore sensitive because: i) N supplies by fertilization might highly differ from the soil N-available, leading to an overestimation of NUE in most of situations, especially in lower N-applied situations and to an underestimation of NUE in overfertilized N situations (Cormier et al., 2013), ii) the N-limited regimes definition could exhibit a huge range of variation between studies. Therefore, a reduced N input might not necessarily represent a limiting N-constraint for plant growth, in particular if the soil residual N and/or net N mineralization are high.

To evaluate the impact of N-availability on crop growth, the Nitrogen Nutrition Index (NNI) could be used. This index characterizes crop N-status and has been used to study NUE in major crops (Dordas et al., 2011). The NNI (Eq. 10) was defined by Lemaire and Salette (1984) as the ratio of the N content at a given above-ground biomass to the critical N-concentration corresponding to this given above-ground biomass. It is defined by the equation:

$$\text{NNI} = \text{Nt} / \text{Nc} \quad \text{Equation 10.}$$

; where Nt is the total N concentration measured in the shoot, and Nc the critical nitrogen concentration corresponding to the same shoot biomass. When NNI values are equal to 1, the plant N status is considered as optimum; NNI values higher than 1 indicate an excess of N and values lower than 1 indicate a N constraint. The critical N concentration is defined as being the minimum plant N concentration allowing maximum growth rate (Ulrich, 1952). The estimation of critical N concentration (Eq. 11) for different level of shoot dry mass allows determining a critical dilution curve as follows:

$$\%N_c = a_c W^{-b} \quad \text{Equation 11.}$$

; where a_c is the critical plant N concentration, W is the total shoot biomass (t ha^{-1}) and b is a positive constant.

The critical N-concentration was defined generically for C3 plants by Lemaire and Gastal (1997) and specifically for oilseed rape by Colnenne et al. (1998) (Figure II.1). While Lemaire and Gastal (1997) proposed a curve valid for biomass higher than 1 t ha^{-1} , Colnenne et al. (1998) considered a constant critical N concentration for above-ground biomass less than 1 t ha^{-1} , corresponding to early growing stages, before any competition for light between plants.

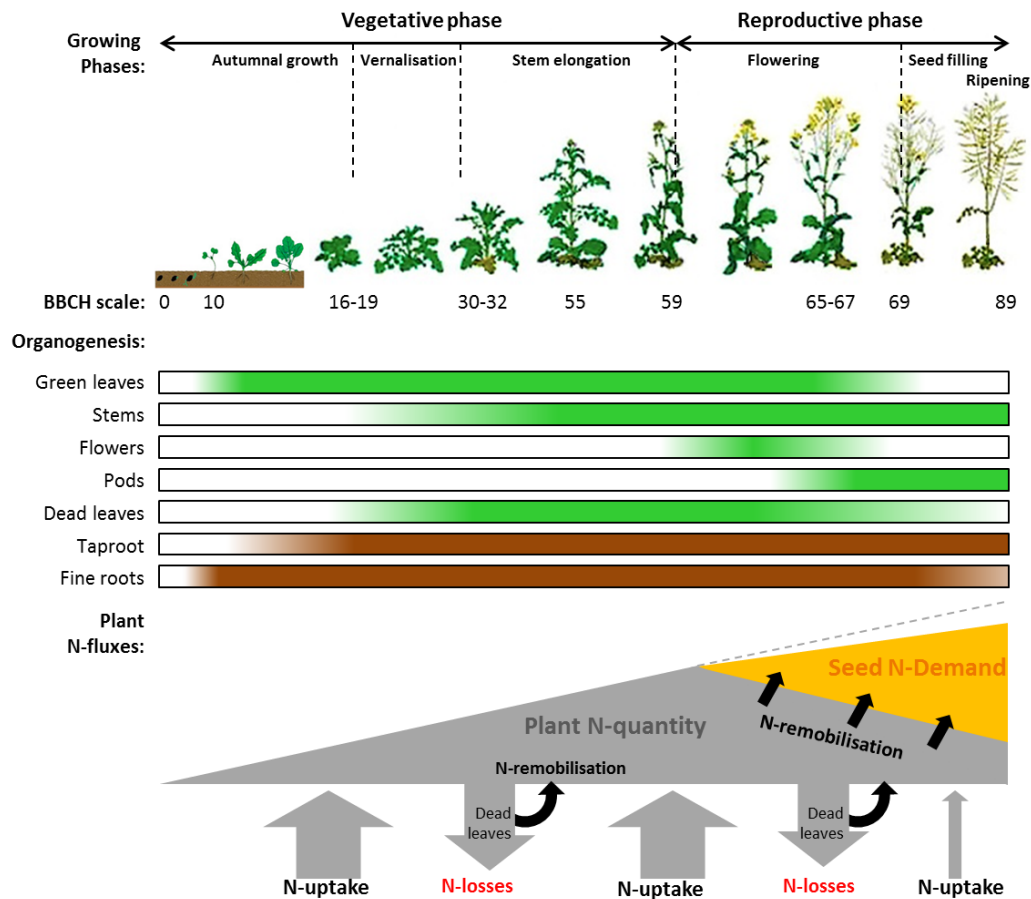


Figure II.2. Conceptual diagram of winter oilseed rape development and N functioning during the growing cycle. BBCH scale according to Lancashire et al., 1991. Organogenesis: green shaded bars depicts the contribution of above-ground organs to whole-plant dry matter for a given growing phase, whereas brown shaded bars correspond to below-ground organs. Plant N-quantity (lower part of the figure) results from (1) pre-flowering N-uptake from N available in soil (including N absorbed from mineralization of fallen leaves during crop cycle) and N-remobilization from senescent leaves to produce vegetative biomass, (2) post-flowering N-uptake (grey dotted line depicts a potential N-pool available from a late N-uptake after flowering), to maintain an N pool in the plant during the pod development and seed filling period, and (3) N-remobilization between organs to ensure seed N-filling. Arrows depicts N-fluxes. Adapted from Alexandra Jullien (personal communication).

However, after flowering, the allometric relationship between N concentration and shoot dry weight changes as the plant ages (loss of leaves, increase in lignified tissues) and also with changes in the biochemical nature of storage materials such as lipids in oilseed rape. Indeed, the NNI should be used until flowering, but not after. Although the N-dilution curve proposed by Colnenne et al. (1998) was calibrated until flowering stage on above-ground biomass measures without including fallen leaves, this curve remains the standard for oilseed rape studies.

To sum up, the definition of NUE given by Moll et al. (1982), defined as the ratio of seed yield to N supply to the crop, is the most commonly used by agronomists for evaluating crop N-requirements. However, this definition does not reflect the underlying physiological processes of plant response to N-availability.

From an ecophysiological point of view, considering the ratio of total biomass produced to the total amount of N-available during the growing cycle seems relevant to evaluate NUE dynamically. The quantification of the total plant N-amount (including dead leaves N-losses and N-amount in the root system) and the accurate estimation of the N-available in soil are two main issues when discussing for plant and genotypes responses to N-conditions, specially under low-N conditions.

Last, to study crop response to N-limitations, the NNI seems useful to assess if N-condition leads to a N-constraint limiting for plant growth or not. However, although the Colnenne et al. (1998) N-dilution curve has been widely used in oilseed rape, it does not account for fallen leaves, hence it might introduce a significant bias in winter oilseed rape.

2.2. Processes related to nitrogen use efficiency in oilseed rape

To better understand the process related to N-use efficiency in winter oilseed rape, a thorough knowledge of the development and growth of aerial and root organs during the crop cycle is required. Improving N-efficiency in oilseed rape requires a good understanding of N demands of the different organs at the successive growth phases and development stages (Li et al., 2016). Indeed, NUE results from the interaction among processes at the canopy level (i.e. leaf area dynamics, biomass accumulation and light interception) and at the plant level (i.e. N-uptake, leaf senescence and N-C fluxes across the plant). Those processes should be studied at the whole plant level, integrating C-N source-sink dynamics (Xu et al., 2012), and accounting for the architectural plasticity modulated by plant interactions at the canopy level.

2.2.1. The oilseed rape plant: development, growth and N-metabolism

Oilseed rape is a member of the genus *Brassica* within the family *Brassicaceae*. It results from a spontaneous interspecific hybridization between *Brassica rapa* and *Brassica oleracea* (Nagaharu, 1935). Oilseed rape presents a strong eco-geographical differentiation into spring varieties and winter varieties. This differentiation is under the genetic control of mechanisms that regulate the vernalisation requirement and onset of flowering.

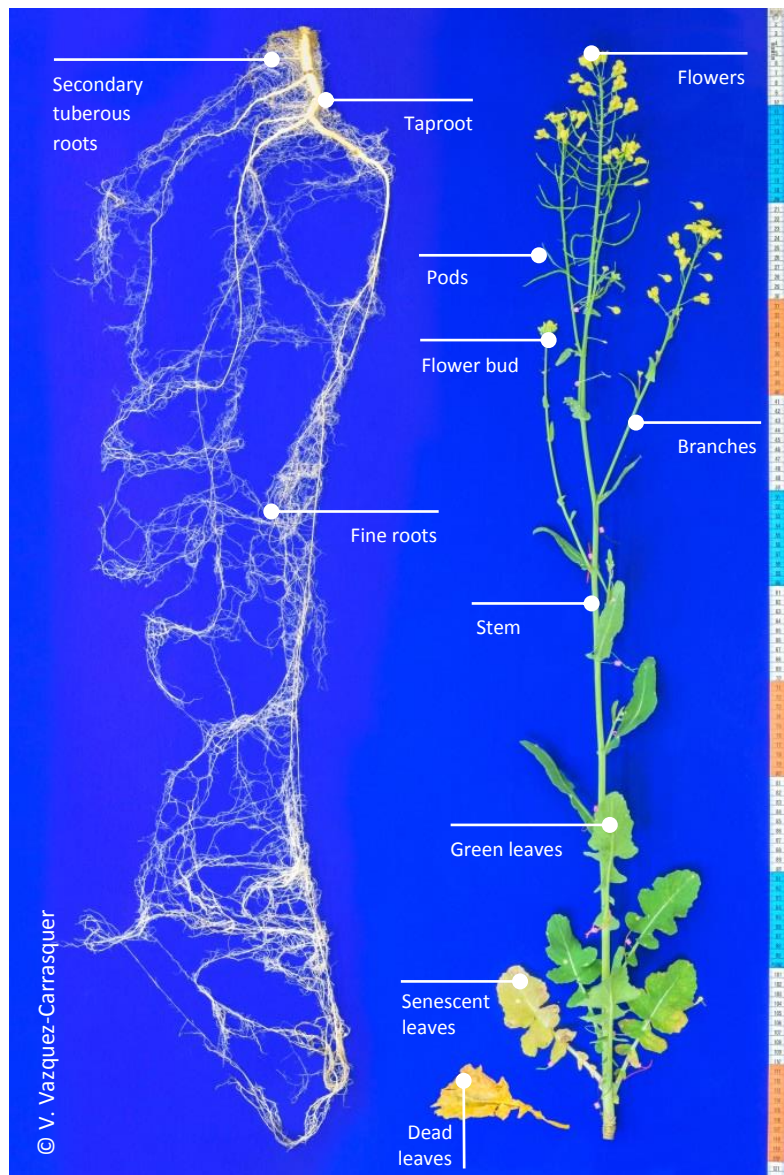


Figure II.3. The oilseed rape plant: shoot and root organs at the flowering stage.
Scale colored bands correspond to 10 cm.

Winter oilseed rape is the predominant form in Europe; hence this literature review has been focused on it. In temperate climates, winter oilseed rape is typically sown in late August or early-to-mid September. In Europe, it is typically sown in a density of 50–120 seeds per m² (Liu et al., 2019), to achieve a plant density of 35–45 plants per m² after winter (Edwards and Hertel, 2011). The plant flowers in spring (March to May) and the crop is generally harvested in end of June or July and up to August. The growing cycle varied from 270 to about 300 days, as the plant needs to accumulate around 2400 degree-days from sowing to seed maturity, with a base temperature of 0°C (Hebinger, 2013), to complete the growing cycle. Although a base temperature of 0 °C has been mainly used in winter oilseed rape, some studies used base temperatures ranging from 3 to 5°C (Leterme, 1988; Marshall and Squire 1996; Justes et al., 2000; Jullien et al., 2011; Brunel-Muguet et al., 2015; Villar et al., 2019; Liu et al., 2019).

Growth and development of the winter oilseed rape is a complex process because some of the main stages overlap widely, resulting in a complex plant architecture (Figure II.3), as described at the crop (Leterme, 1988) and the plant scale (Jullien et al. 2011). The international BBCH system is generally used to describe oilseed rape phenology (Lancashire et al., 1991) (Figure II.2). It subdivides the whole plant cycle into nine main phases, with five phases during the vegetative growth (0. Germination; 1. Leaf development on main stem; 2. Formation of side shoots; 3. Stem elongation, 5. Flower bud emergence) and four phases during the reproductive period (6. Flowering; 7. Development of pod; 8. Ripening of pod; 9. Plant Senescence). Each phase is subdivided into secondary growing stages.

2.2.1.1. Vegetative growth

Vegetative growth extends from seedling emergence (BBCH 00–09) up to flowering starts (BBCH 60), through growth cessation during winter, and stem elongation (BBCH 30–39) and flower bud emergence (BBCH 50–59) in spring (Figure II.2). This is the longest growth phase of the cycle (Marjanovic-Jeromela et al., 2019). During the autumnal growth (from BBCH 00 to 29), the plant biomass increases (up to 3 t ha⁻¹) and N is efficiently uptaken from the soil and stored into the vegetative biomass (Edwards and Hertel, 2011), with up to 100 kg N ha⁻¹ being absorbed before winter (Rossato et al., 2001; Dejoux et al. 2000).

The root system develops during this period to reach a maximum size between late-flowering and late-pod stages and then decreasing to maturity (Gan et al., 2009). It is composed of taproots, which act as a reservoir for nutrients and assimilates, and fine roots, supporting water and nutrient uptake and presenting ability to branch and proliferate in zones of higher nutrient content (Edwards and Hertel, 2011). Taproots account for 80 to 85% of the total root biomass in the field studies (Kjellstrom and Kirchmann 1994), but only 9% of the total N-absorption process (Rossato et al., 2001). The root system responds to environmental conditions, and its net growth results from root production and root mortality (Kamh et al., 2005). Nitrate (NO₃⁻) is the predominant form of N-absorbed (Rathke et al., 2006). Nitrogen absorption is mainly driven by plant's demand and by N availability in the soil, which modulates the N absorption rate per unit root length (Dreccer et al., 2000; Kamh et al., 2005). Thus, N absorption can be limited by the root development or by the soil N and/or water availabilities (Gallais and Hirel, 2004).

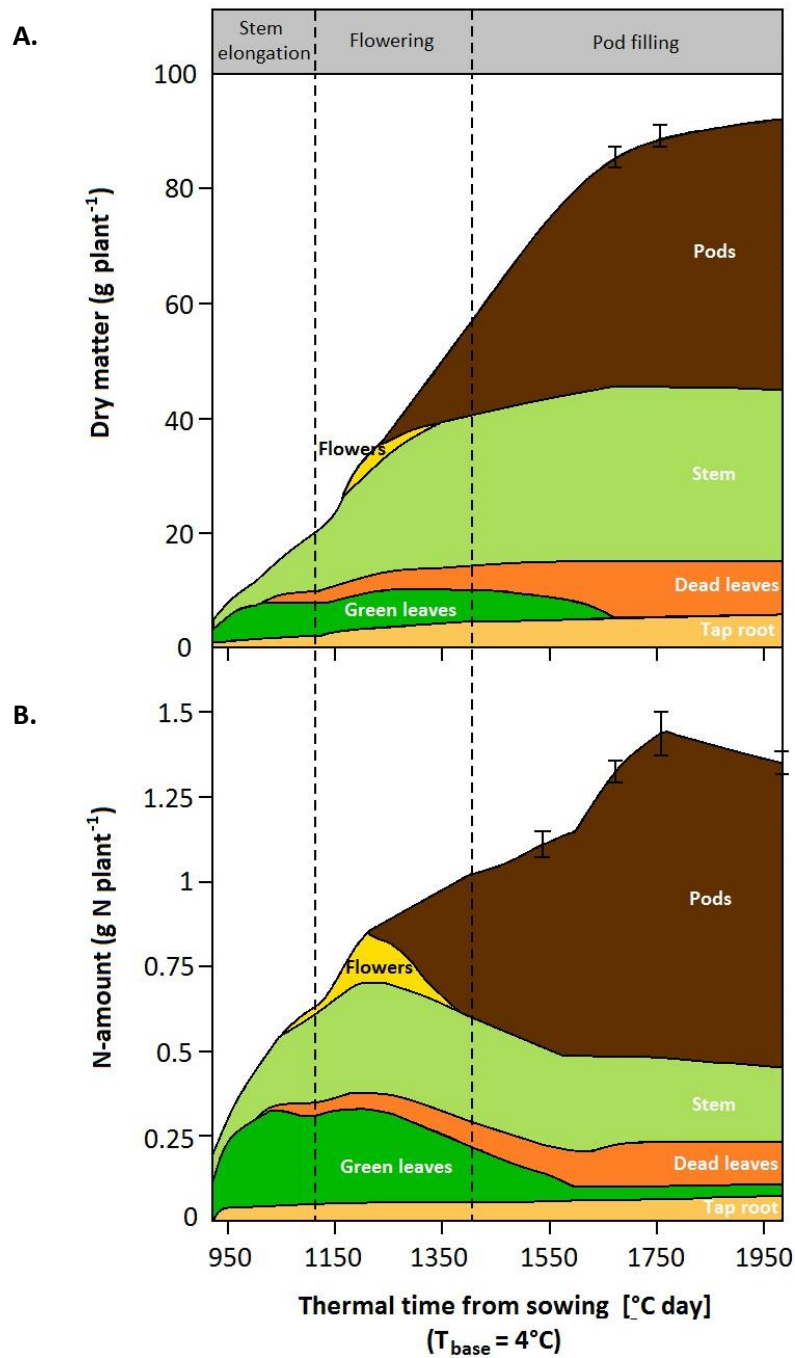


Figure II.4. Thermal time courses of plant biomass (A) and N-amount (B) in winter oilseed rape (cv Capitol) growing under non-limiting N-conditions (225 kg N ha^{-1}), and their repartitions between organs. Colors correspond to plant fractions: taproot (light orange), green leaves (green), fallen leaves (orange), stem (light green), flowers (yellow) and pods (brown). Vertical bars indicate the standard deviation ($n = 3$) when larger than the symbol. Adapted from Malagoli et al. (2005a).

N uptake can be understood in a narrow sense as the active transport process to carry N over the plasmalemma membrane into the cell interior (Lainé et al., 1993). This process, also called specific N uptake (SNU), is achieved by nitrate and ammonium transporters (reviewed in Xu et al., 2012). In a broader sense, N uptake includes morphological properties of the root system (*i.e.* soil exploration) as well as the efficiency properties of the root system *per se* (*i.e.* SNU expressed by N uptake per root area unit) (Kamh et al., 2005).

In autumn and winter, under non-limiting N-conditions, the decrease of leaf area index (LAI; Watson, 1947), can limit the evolution of the radiation interception efficiency (RIE) (Monteith, 1977). Simultaneously, N is remobilized from older to younger leaves due to sequential senescence, supporting the initiation of new foliar primordia as early as the end of autumn as well as the determination of the potential reproductive organs, that will grow later in spring (Robelin and Triboi 1983). Indeed, the numbers of leaves, branches, flowers, and ovules are partly determined before the end of winter (Diepenbrock, 2000; Allirand et al., 2011). During the overwintering period, plants lose a large number of leaves, causing N losses estimated to approximately 20 kg N ha⁻¹ (Rathke et al., 2006; Malagoli et al., 2005), rising up to 100 kg N ha⁻¹ according to Dejoux et al. (1999). The loss of leaves is influenced by abiotic stresses such as flooding, freezing or reduced incoming radiation. Moreover, RUE can be reduced by a N-deficit as well as by temperatures below 6–7 °C (Justes et al., 2000). The organic N in the fallen leaves will be mineralized later, supplying a potential pool of mineral N re-absorbed partly by the crop during the spring. Dejoux et al. (2000) quantified the net mineralization of lost leaves from 20 up to 40 kg N ha⁻¹. Villar et al. (2019) reported an increase of nitrate leaching due to a net mineralization at the end of the oilseed rape crop cycle up to 21 kg N ha⁻¹, a nitrate leaching up to 7 kg N ha⁻¹ and N₂O emissions up to 1.6 kg N ha⁻¹.

After winter, plant's growth starts again depending on the length of the photoperiod and when the daily temperature regularly exceeds 4.5 °C (Rathke et al., 2006; Böttcher et al., 2016). The reestablishment of the crop in spring is closely related to the storage of C and N reserves in plants during autumn and winter (Tian et al., 2017). The environmental factors determine the beginning of the main stem elongation, which overlaps with leaf expansion (Edwards and Hertel, 2011). This is a very active period for organ expansion that accounts for biomass accumulation, with about 80 % of the total plant biomass constituted by green leaves and the stem (Malagoli et al., 2005) (Figure II.4A), and an essential period for the N-absorption (Gabrielle et al., 1998). Indeed, leaves represented the first major sink for nitrogen in spring, followed by stems (Figure II.4B) (Malagoli et al., 2005; Bissuel et al., 2019). As the stems elongate, the roots continue to grow deeper and to extend laterally, and the plant stores the excess of N and C assimilates in taproots, leaves and stems (Wright et al., 1988). Indeed, the accumulated biomass before flowering has a major impact on final yield elaboration, and highly determines the NUE (Yau and Thurling, 1987; Stahl et al., 2019).

2.2.1.2. Reproductive phase

The reproductive phase overlaps with the end of the stem elongation. It begins when the first flowers open on the main stem (BBCH 60-61) and extend through the pod development (BBCH 71-79) up to seed maturity (BBCH 89) (Figure I.2).

However, the reproductive organs develop from floral initiation, the flower buds start to grow in winter and remain enclosed in the leaves (Edwards and Hertel, 2011). Flowering is the most critical stage influencing oilseed rape yield (Diepenbrock, 2000), as seed yield potential is set by the balance between biomass elaboration and N-uptake during the vegetative growth and the potential number of flowers, pods and seeds (Edwards and Hertel, 2011). From the beginning of flowering (BBCH 60), there are no more leaves produced on the main stem (Justes et al., 2000) (Figure II.4A), and the potential number of lateral branches and subsequently the potential numbers of pods and seeds are largely fixed (Rathke et al., 2006). Increases in branches number leads to higher number of pods on the branches and, as a consequence, to higher seed yield (Stahl et al., 2019). At flowering, only 40% of the solar radiation reaches the leaves (Robelin and Triboï, 1983), as a large proportion of PAR is either reflected or shaded by the flower layer (Justes et al., 2000; Dreccer et al., 2000). As a consequence, the photosynthesis decreases and leaf senescence increases (Rathke et al., 2006; Desbøll et al., 2016). This temporary lack of photosynthesis leads to a reduction in the potential seed number by abortion of flower buds and young pods. During this period, N is mainly shared between leaves, stem and flowers, which constitute 35, 35 and 15 % of total N in the plant, respectively (Figure II.4B) (Malagoli et al., 2005).

After flowering, the period of intensive pod development coincides with a reduction in leaf expansion and acceleration of their senescence and falling off (Leterme, 1985; Jullien et al., 2011). Although pod envelopes and stems are known to be photosynthetic organs (Leterme, 1985; Müller and Diepenbrock, 2006; Edwards and Hertel, 2011), pod and stem photosynthesis does not entirely compensate for leaf photosynthesis (Leterme, 1985; Diepenbrock, 2000). The reduction of the photosynthesis leads to a high rate of ramification abortion and to a reduction of the number of pods per plant, suggesting that carbon demand is higher than offer (Jullien et al., 2011). Pods also function as C and N storage organs, which are then redistributed to the developing seeds. Thus, the leaves, stem and taproot act as sources of C and N at flowering and during pod development and seed filling. Therefore N issued from remobilization from vegetative tissues, particularly from leaves, is the main source to face N requirement during pod filling (Malagoli et al., 2005, Dreccer et al., 2000; Rossato et al., 2001). However, the high N concentration remaining in the fallen leaves (Schjoerring et al., 1995; Dreccer et al., 2000; Rossato et al., 2001) suggests an incomplete N remobilization (Dejoux et al., 2000; Malagoli et al., 2005; Gombert 2010).

Several studies indicate that low N-uptake occurs from flowering to pod ripening (Wright et al., 1988; Gabrielle et al., 1998; Malagoli et al., 2005b; Rossato et al., 2001), and N-uptake can even be stopped (Rossato et al., 2001). In all cases the N-uptake *per se* after flowering is not sufficient to meet the total N demand of the reproductive organs (Masclaux-Daubresse et al., 2008). However, the N-absorbed during this period may sustain the plant N pool available, maintaining the global functioning of the plant, and might be remobilized later in pods and seeds (Malagoli et al., 2005; Bouchet et al., 2016).

At maturity, dehydrated seeds change from green to black color. The oil concentration of the seed increases, being maximized by the time 40% of the seeds have changed color. When 60% of the seeds have changed color, the maximum seed dry weight has been reached. Seeds are fully mature (physiological maturity) about 950-1000 degree-days after flowering (Leterme 1985, 1988; Griffith, 2013; Jullien et al., 2011). Indeed, biomass allocation into seeds in a given N-condition will determine the N-use efficiency of the crop.

2.2.2. Interaction between carbon and nitrogen metabolism and between shoot and root compartments

Crop carbon assimilation is related to crop N through leaf area development and nitrogen distribution between leaves with consequences for canopy photosynthesis (Gastal and Lemaire 2002). Therefore, crop growth fundamentally relies on the balance of N allocation between growing and fully expanding leaves. Indeed, crop growth depends on the interactions between the nitrogen and carbon balance involving photosynthetic processes such as leaf area index (LAI), pod area index (PAI), and radiation use efficiency (RUE) (Lemaire and Salette 1984).

The amount of carbon allocated into roots may limit nitrate uptake during leaf expansion (Tolley-Henry et al. 1988). High nitrate uptake might contribute to an enhanced N accumulation in young leaves, maintaining a higher photosynthetic capacity and delaying leaf senescence (Schulte Auf'm Erley et al. 2007). Indeed, the remobilized N from the leaves may be used to increase the photosynthesis rate of the pods, which contribute significantly to carbon gain of oilseed rape after flowering (Gammelvind et al. 1996) or to avoid a reduction in pod number that is enhanced by N deficiency (Hocking et al. 1997) and appears to be related to N remobilization from vegetative plant parts (Schjoerring et al. 1995). Indeed, Schulte auf'm Erley et al. 2007 pointed out that the most N-efficient oilseed rape genotypes were those adapting leaf photosynthetic capacity to the low-light conditions in the canopy during flowering. Koeslin-Findeklee et al., 2014 showed that prolonged N-absorption during the reproductive phase might extend leaf-photosynthesis duration maintaining the photosynthetic capacity of leaves (in particular leaves at lower canopy layer) through delayed leaf senescence. This should result in a higher C-pool for the pods but also for the roots (Koeslin-Findeklee et al., 2014). A prolonged assimilate allocation to the roots could maintain the root growth and consequently the N uptake duration (Osaki, 1995).

Nitrogen uptake and distribution also depends on C allocation between organs (Gastal and Lemaire 2002). Shoot biomass increases in relation to C allocation to the leaves and stems over the two crop growth periods. Both quantities and allocations of C and N between shoots and roots also change in relation to soil N availability and/or crop N uptake and C assimilation. Regulation of nitrogen assimilation needs to be considered in the context of these interregulatory processes. Indeed, several studies have shown that root growth and the emergence of new roots are highly sensitive to carbon availability. Brun et al. (2010) showed that carbon flux originating from the shoot explained the major part of root system adaptation to nitrogen availability in *Arabidopsis thaliana*. Moreover, Moreau et al. (2017) pointed out that differences in root system architecture in soils with contrasted nitrogen availability are mainly due to differences in the amount of carbon allocated to and within the root system.

Furthermore, some studies have demonstrated that plants can uptake and assimilate more N under a high CO₂ atmosphere (Stitt and Krapp, 1999, Andrews et al., 2001), suggesting that enzymes involved in N metabolism do not generally limit biomass elaboration in plants (Andrews et al., 2004). Therefore, to improve N-uptake under low N-availability targeting other processes (e.g. N-uptake efficiency, C-N allocation to root system) than N metabolism enzymes and focusing on traits contributing to root system architecture might be more relevant.

To sum up, the oilseed rape growing cycle can be split into two main periods: vegetative growth and reproductive period. However, oilseed rape growth and development is complex because i) some of the main phenological stages overlap widely and ii) autumn and spring are two distinguished periods of biomass accumulation and N uptake followed by a long period of N remobilization, pod accumulation and seed filling.

Green leaves represented a first major sink, followed by stems, for N and C assimilates. However, N remobilization before leaf fall is relatively low in winter oilseed rape, and might impact the potential pool of N-available after flowering. Indeed, N-uptake per se seems insufficient to meet the total N demand of the reproductive organs. A prolonged N-uptake and an improved N remobilization after flowering are essential to maintain the plant's global functioning, especially under low-N conditions.

Improving oilseed rape N-efficiency requires a good understanding of its C and N demands during the growing cycle as well as a thorough knowledge of the dynamics of biomass elaboration, especially of the root system, which remains relatively unexplored. Indeed, although taproots account for up to 85% of the total root biomass, they only accounted for 9% of the total N-absorption, pointing up the role of fine roots in N-absorption and the interest of going deeper in below-ground trait's research, especially in a context of lower N-inputs.

2.3. N-availability has major role on oilseed rape functioning

2.3.1. Oilseed rape N-requirements

Oilseed rape growth is highly sensitive to soil moisture and N-availability (Colnenne et al., 1999; Albert et al., 2012; Balodis et al., 2015). Oilseed rape needs around 6.5 to 7 kg of nitrogen absorbed per quintal of seed produced. Above 300 N-units absorbed, nitrogen does not limit seed yield anymore (Terres Inovia, 2019). However, plant competition for N resources might lead to plant N deficiencies, especially during the period between fertilizations (Lammerts van Bueren et al., 2017). The Terres Inovia Institute recommends a fractioning of N supplied in up to three N-fertilizations accordingly for: fertilizations lower than 100 kg N ha⁻¹, a single fertilization at the development of the main inflorescence (BBCH 51-53) is recommended; for fertilizations ranging from 100 to 170 kg N ha⁻¹ a first fertilization at the end of the stem elongation (BBCH 39-51) and a second fertilization at BBCH 51-53 are required; and for fertilizations higher than 170 kg N ha⁻¹ a first fertilization at the beginning of the stem elongation (BBCH 31-32), a second fertilization between BBCH 39 and BBCH 53 and a third fertilization at just before flowering (BBCH 59) are recommended. However, N-input should be fine-tuned to the temporally changing requirements of the crop, as N demand varies with growth stage and with plant's biomass accumulation (Gabrielle et al., 1998; Wiesler et al., 2001), as well as on the environmental factors. Indeed,

Heavy rainfalls along with high doses of N-fertilizer lead to leaching of nitrate (Gammelvind et al., 1996), besides waterlogging during autumn/winter affects growth, N-uptake, and yield formation (Zhou et al., 1997). In contrast, water deficit reduces soil N-availability, due to a lower effectiveness of mineral N-fertilizer or to reduced mineralization of soil organic N, as well as reduced N-absorption capacity of roots (Steudle, 2000). Moreover, nitrogen has to be available in the space where crop roots can take it up (Lammerts van Bueren et al., 2017). Indeed, Gastal and Lemaire (2002) suggested that for crops growing under low N-availability, N-uptake mainly depends on root's distribution over the soil profile. In contrast, under optimal or high N-availability, N uptake depends more on growth rate via internal plant regulation (*i.e.* N-utilization processes). However, Gabrielle et al., (1998) highlighted that although N-limitations strongly alter oilseed rape shoot growth, they did not significantly affect the dynamics of root growth at depth. Moreover, root profiles carried out in the field under low and high N conditions for rapeseed also showed that root depth was even higher under limiting conditions (Albert et al, 2008).

As the current and future agricultural context aims at reducing N-inputs, we focused the literature review on the impacts of N-limitations on NUE-related processes and whole plant traits.

2.3.2. Impact of N-limitation in NUE-related processes

N-availability modulates the relative contribution of N-uptake efficiency (NUpE) and N utilization efficiency (NUE) to the overall NUE. Indeed, several studies investigated the correlations between NUE and NUpE or NUE at harvest, under contrasting growing N-conditions.

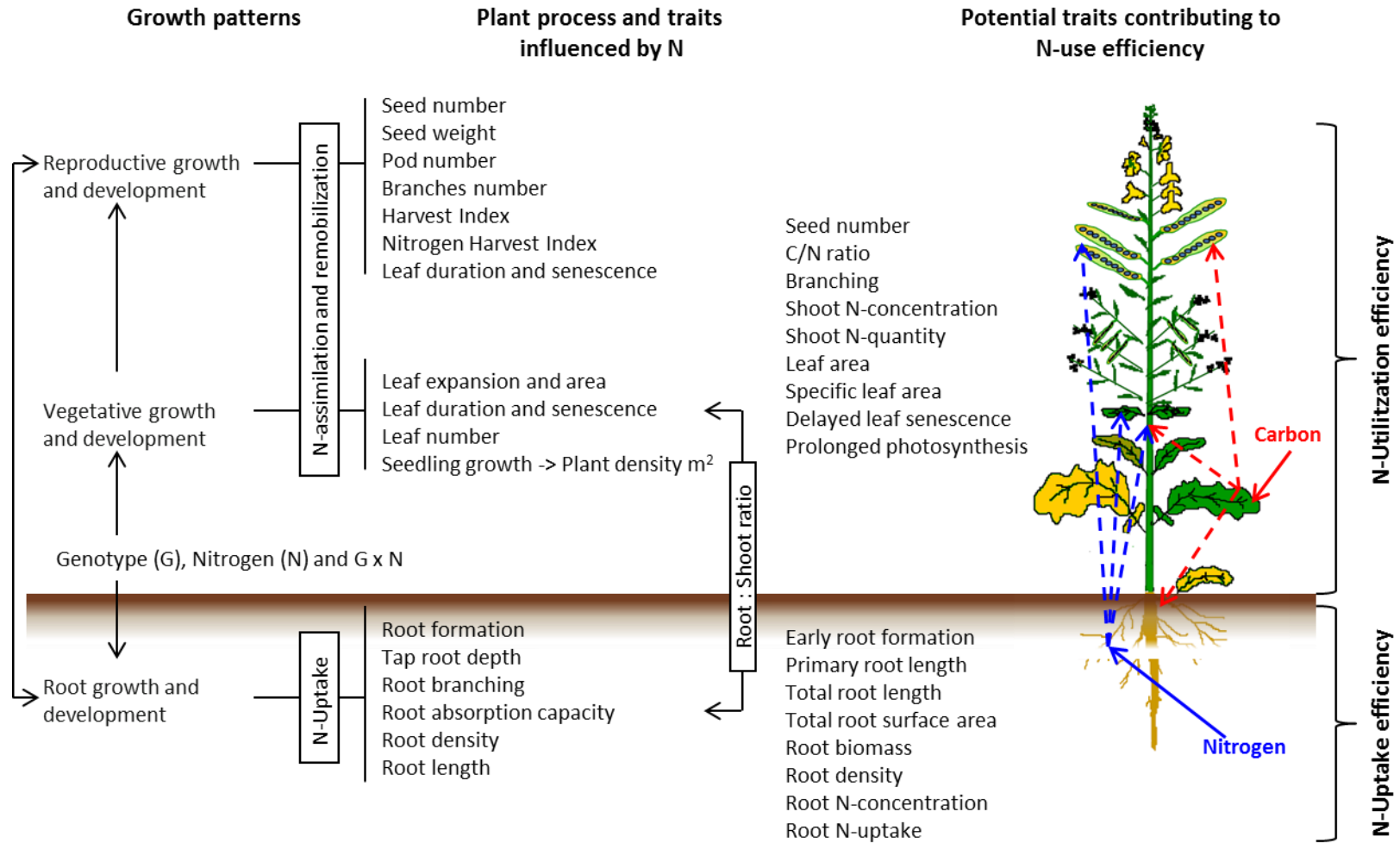


Figure II.5. Plant processes and traits influenced by nitrogen availability and contributing to NUE in oilseed rape. Figure depicts C-N partitioning between roots, leaves and pods with seeds. Adapted from Lammerts van Bueren et al. (2017).

Several authors have investigated the correlation between NUE and NUpE and NUtE at seed maturity and under contrasting N-conditions. Under high-N conditions, variation in NUE was mainly correlated to variation in NUtE (Berry et al., 2010; Kessel et al., 2012; Koeslin-Findeklee et al., 2014; He et al., 2017). Under low-N conditions, however, variations in NUE were more correlated to variations in NUpE (Berry et al., 2010; Schulte auf'm Erley et al., 2011; Kessel et al., 2012; Miersch, 2014; Nyikako et al., 2014). Therefore, the enhancement of oilseed rape performances under low N-availability would primarily require the improvement of the NUpE, as reported in wheat (Gaju et al., 2011), rice (Rakotoson et al., 2017) and maize (Li et al., 2015). In contrast to these studies, Svecnjak and Rengel (2006b) found that correlations with NUE are much lower for NUpE than for NUtE, whatever the N-condition. However, these results should be used with particular care as the experiments were performed in small pots and root growth might have been influenced by the reduced soil volumes and the higher soil temperatures of pot experiments than in a field context, as stressed by Poorter et al. (2012).

Plant processes and traits influenced by nitrogen availability are summarized in Figure II.5.

2.3.3. Impact of N-limitation on plant traits at canopy level

Several authors reported a negative impact of low-N availability in oilseed rape seed yield (Schjoerring et al., 1995; Hocking et al., 1997; Barlog and Grzebisz, 2004; Chamorro et al., 2002; Rathke et al., 2005; Stahl et al. 2019). Low-N availability during autumn and spring development reduces the LAI expansion and duration (Lemaire et al., 2008; Justes et al., 2000), resulting in a reduction of the radiation use efficiency (RUE) (Lemaire et al., 2007). Reduced C assimilation is correlated with lower dry matter accumulation by the crop and a limited number of ramifications and pods, resulting in lower seed yield (Allen and Morgan, 1972).

Moreover, low-N availability might affect crop establishment hence reducing crop plant density. Low-N availability also decreases the number of branches and therefore the number of seeds per square meter, which is the main component of seed yield (Bissuel et al., 2011). Nevertheless, oilseed rape crops have a large plasticity to compensate for low-plant density by increasing branching (Allirand et al., 2011; Jullien et al., 2011), the number of seeds per branch, and the number of seeds per pod (Pinet et al., 2015). However, these studies have been conducted under non-limiting N conditions. Moreover, N limitation reduces the number of seeds per m², mainly on the lateral branches, where the number is reduced (Bissuel et al., 2019). It has been recently demonstrated that plasticity of seed weight (TSW) can compensate for deficiencies in other yield components (Labra et al., 2017). However, this trait is genotype-dependent and weakly explains the observed variation in seed yield and NUE in response to field N-conditions (Bissuel et al., 2019). Moreover, the amount of N effectively remobilized from the vegetative parts to the seeds during the seed filling period is conditioned by the pool of N-available in the aerial biomass (Stahl et al., 2015).

2.3.4. Impact of N-limitation on plant traits at the plant level

Low-N availability reduces seedling growth that is closely related to the establishment of the sink strength (Li et al., 2016). Nevertheless, the main ecophysiological processes underlying NUE in oilseed rape at the plant level include (1) pre-winter and pre-flowering N-uptake and N-remobilization, to support production of photosynthetic leaf area and vegetative biomass, (2) post-flowering N-uptake and N-remobilization, to sustain the N pool in the plant during the pod development and seed filling, (3) and N-remobilization associated with senescence processes from flowering onwards, to complete maximal remobilization of the N from the pod wall into the seeds (reviewed in Bouchet et al., 2016) (Figure II.2).

Leaf senescence is a mechanism tightly controlled by N availability (Abdallah et al., 2011). Indeed, Girondé et al., (2014) highlighted that an enhanced N remobilization during vegetative growth contributed improving NUE by reducing the N loss by leaf drop, but this effect needs to be associated with efficient utilization of the remobilized N in young tissues to improve leaf growth. Under low-N conditions, Schulte auf'm Erley et al., (2007, 2011) suggested that delayed leaf senescence, contributed to continued root activity and N uptake, considering high efficiency in N uptake up to flowering (leading to high N accumulation in young leaves) as essential. However, Koeslin-Findeklee et al. (2014), found that oilseed rape N-uptake dynamic was not modified by late senescence.

The apparent rate of N remobilization from leaves to pods is similar independently of the considered N fertilization (Gombert et al., 2010). This led to more N found in fallen leaves at high N fertilization levels, indicating that N remobilization is sink-limited under high-N conditions. An alternative explanation for the higher N concentration in fallen leaves under high N fertilization was suggested to be related to the N repartition in the leaves. Structurally bound N is less likely to be remobilized, and leaves with a higher N concentration also have a higher proportion of structurally bound N, this might explain the higher N content of the fallen leaves from the high N treatments (Ulas et al., 2013). Furthermore, Allirand et al. (2007) suggested that leaves of plants grown under high-N conditions exhibited higher leaf area, then lower leaves were more shaded, as above leaf area intercepts more radiation compared to plants growing under low-N conditions. As a result, below leaves fall down with higher N concentration, due to the dual control of N-availability and shading.

Regarding N-uptake, [Kant et al. \(2011\)](#) highlighted that nitrate concentration affects root development and root architecture. The root : shoot ratio increases under low-N conditions ([Gastal and Lemaire, 2002](#)). Root traits (*i.e.* taproot depth, fine root density, number of lateral roots, elongation rate, root length and early root growth) are therefore enhanced under low-N conditions ([Svecnjak and Rengel 2006a](#); [Zhu et al., 2011](#); [Koscielny et al., 2012](#); [Aibara and Miwa, 2014](#); [Rao et al., 2016](#); [Li et al., 2016](#); [He et al., 2017](#)). Nevertheless, [Colnenne et al., \(2002\)](#) highlighted that tap root is less sensitive to low-N conditions than the aerial parts. [Hirel et al. \(2007\)](#) pointed out that further work is necessary to ascertain the role of root system in the elaboration of NUE and its components, taking into account the oilseed rape specificities in terms of NUpE and duration of N uptake before and after flowering ([Hirel et al., 2007](#)).

Under low-N conditions, it might exist a scope of improvement of NUE by i) better managing the N-inputs in space and time and ii) by improving the N-uptake efficiency.

At the canopy scale, N-limitation has a negative impact on crop growth, which could be observed both on processes (e.g. photosynthetic leaf duration, N-uptake) and on integrative variables (e.g. shoot biomass, LAI, seed yield). Moreover, due to the high canopy plasticity and the interacting and compensation processes at the canopy scale, it seems relevant to go down to plant's level to assess the underlying processes of oilseed rape responses to N-conditions and decipher integrative responses observed at the canopy level.

At the plant scale, low-N availability impacts plant's growth from seedling stages, affecting both the establishment of the surfaces and the potential sink strength of different organs. Moreover, leaf senescence is a major process linking the C and N metabolisms and is tightly controlled by N availability. Last, the root: shoot ratio increases under low-N conditions and root traits such as root depth and density are enhanced under low-N conditions compared to the shoot traits.

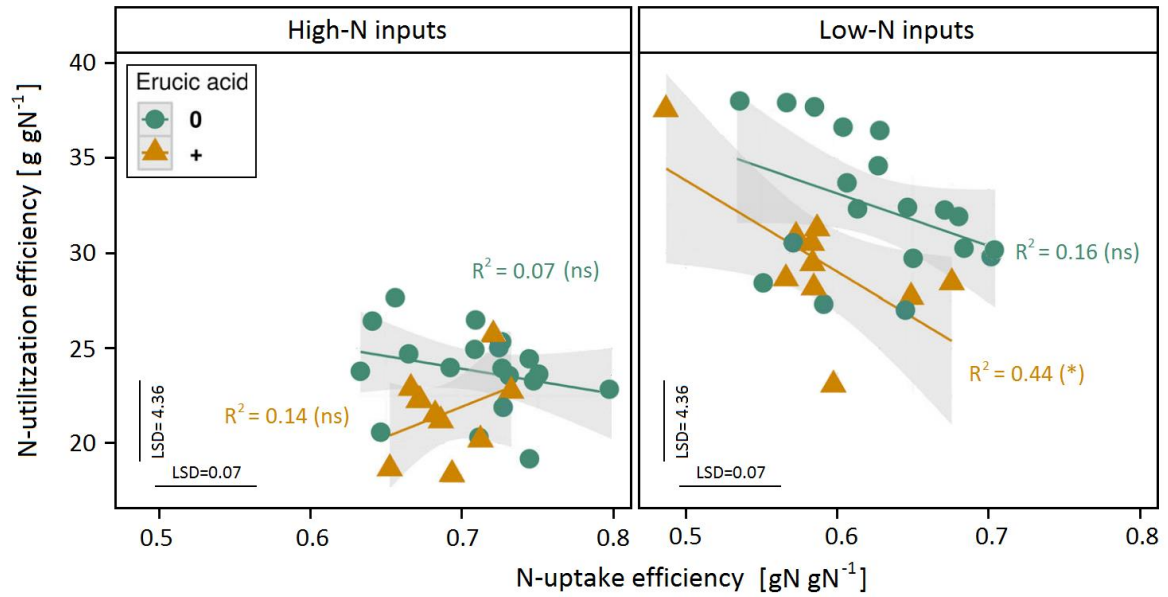


Figure II.6. Relationship of N utilization efficiency (NUE) and N uptake efficiency (NUpE) at harvest at high (left) and low N (right) fertilization inputs, for 30 winter oilseed rape accessions. Colors and symbols correspond to the erucic acid content of the accessions. High-N corresponds to N supply of 2.2 gN plant⁻¹, whereas low-N corresponds to 0.7 gN plant⁻¹. N-utilization efficiency is expressed as the ratio between the seed yield [g plant⁻¹] and the above-ground N-quantity [gN plant⁻¹]. N-uptake efficiency is expressed as the ratio between the above-ground N-quantity [gN plant⁻¹] and the N-inputs from fertilizers [gN plant⁻¹]. The regression equations and coefficients of determination (R^2) are indicated in the figures. Regression significance is indicated for * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Adapted from Stahl et al. (2015).

2.4. Genetic diversity of NUE-related processes and associated traits in response to N-conditions

Potential genetic diversity exists for NUE in winter oilseed rape as suggested by [Berry et al., \(2010\)](#), [Bissuel et al., \(2011\)](#), [Schulte auf'm Erley et al., \(2011\)](#), [Ulas et al., \(2013\)](#), [Bouchet et al., \(2014, 2016\)](#), [Wang et al., \(2017\)](#) and [Stahl et al., \(2017, 2019\)](#). Moreover, several studies evaluated *a posteriori* the consequences on NUE improvement of breeding targeting seed yield ([Berry et al., 2006](#); [Stahl et al., 2017](#); [Stahl et al., 2019](#)), highlighting a genetic progress for NUE concomitant to the breeding efforts carried out for seed yield. However, it might be assumed that breeding schemes directly targeting NUE improvement could enhance NUE-genetic progress ([Kant et al., 2010](#); [Nyikako et al., 2014](#); [Bouchet et al., 2016](#)), as there is a considerable scope for improvement oilseed rape NUE ([Van Bueren et al., 2017](#); [Weiser et al., 2017](#); [Stahl et al., 2019](#)).

2.4.1. Genetic variability of NUE-related processes

Genotypes of winter oilseed rape have different NUE, due to differences in either nitrogen uptake efficiency (NUpE) or nitrogen utilization efficiency (NUtE), usually evaluated at harvest time ([Rathke et al., 2006](#); [Berry et al., 2010](#); [Schulte auf'm Erley et al., 2011](#); [Kessel et al., 2012](#); [Miersch et al., 2016](#)). Therefore, to assess the genotypic variability in response to N, it is needed to study both NUpE and NUtE components. [Stahl et al., \(2015\)](#) did find no genetic correlation between NUpE and NUtE, neither under low nor high-N conditions (Figure II.6), pointing out that these two processes are under different genetic control.

Genetic variation exists for oilseed rape NUpE at both low and high-N conditions. Under low-N conditions, N uptake was the major component explaining genotypic differences in NUE at harvest ([Berry et al., 2010](#), [Schulte auf'm Erley et al., 2011](#)). This is in contrast with the results reported in maize by [Gallais and Coque \(2005\)](#), where genetic variation in NUE at low N-input was related to both components of NUE, specifically nitrogen utilization efficiency, whereas at high N input, it was mainly related to variation in N-uptake. [Dresbøll et al. \(2016\)](#) highlighted that root growth, the N uptake from soil to plant, and the allocation of N in the plant, are processes influence NUE and exhibiting genotypic variation. In oilseed rape, the genotypic variation in N-uptake under low-N occurred mainly after the beginning of flowering ([Schulte auf'm Erley et al. 2011](#), [Kessel et al., 2012](#)). However, if many studies reported genetic variability of NUpE at harvest ([Svečnjak and Rengel, 2006b](#); [Stahl et al., 2015](#)), only a few studies reported it at vegetative stage (e.g. [Svečnjak and Rengel, 2006a](#) at BBCH 18-19).

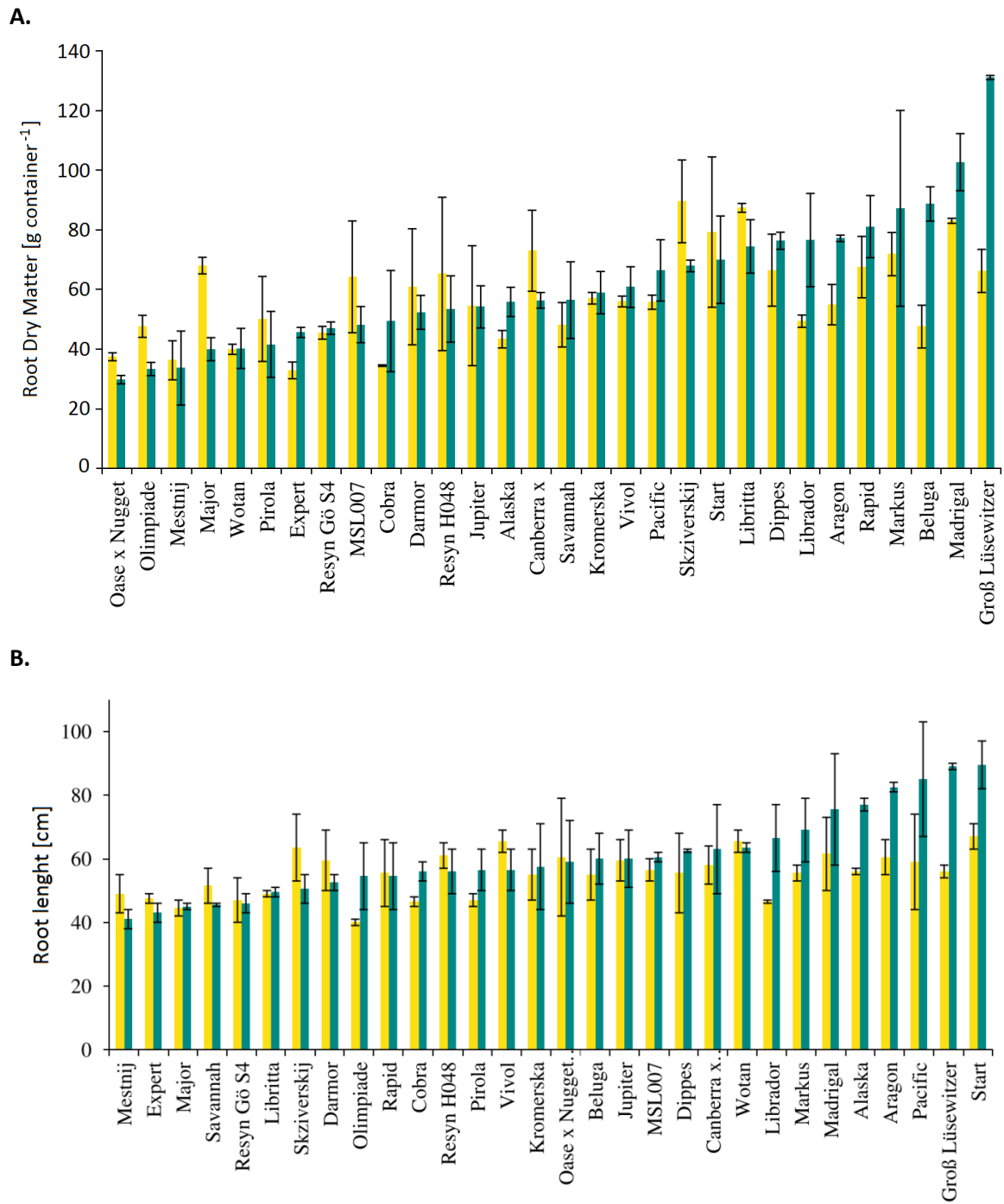


Figure II.7. Genetic diversity for taproot dry matter (A) and taproot length (B) in 30 winter oilseed rape genotypes growing under two contrasted N-conditions in 90 cm deep containers. Data were assessed at harvest. The low-N condition (yellow) corresponds to an equivalent of 75 kg N ha⁻¹; the high N-conditions (green) corresponds to an equivalent of 235 kg N ha⁻¹. Mean values of two replicates are presented, and the standard deviation is marked with error bars. Root length corresponds to the length of the longest root per container. Least significant differences at the 0.05 level were 15.57 cm for root length and 21.25 g for root DM. From Bouchet et al. (2016).

Research to improve NUE in oilseed rape has been mainly carried out on the NUtE component (Malagoli et al., 2005a, b; Gombert et al., 2010; Avice and Etienne, 2014), assuming that the high capacity for N absorption of winter oilseed rape, with up to 100 kg N/ha before flowering (Rossato et al., 2001), would not be the main lever to NUE improvement. Genetic variation has been reported for N-utilization (NUtE) at harvest (Berry et al., 2010; Kessel et al. 2012; Nyikako et al., 2014) and at the vegetative stage (Svečnjak and Rengel, 2006a), as well as for NUtE-associated components: harvest index (Svečnjak and Rengel, 2006b) and biomass production (Yau and Thurling, 1987).

Several studies showed significant correlations between harvest index and NUtE (Berry et al., 2010; Schulte auf'm Erley et al., 2011; Nyikako et al., 2014) under non-limiting N supplies. However, under low-N conditions, these correlations were lower (Schulte auf'm Erley et al., 2011). Wiesler et al. (2001) found that the most N-efficient cultivars under low-N showed better photosynthetic capacity at the end of flowering. N-utilization efficient genotypes exhibited early flowering and were able to synchronize N mobilization with the N demand of the maturing pods, especially at low-N supply (Malagoli et al., 2005; Nyikako et al., 2014; Stahl et al., 2015). However, Bouchet et al. (2016) suggested that early flowering may limit plant's vegetative growth and the amount of N accumulated before flowering, which could lead to a reduced N pool for subsequent remobilization.

2.4.2. Genetic variability of NUE-associated traits

In a 3-year field study, Ulas et al. (2012) found that N-efficient genotypes could be characterized by higher root biomasses during the vegetative stages. Genetic diversity of root biomass, root length and root/shoot ratio was pointed out at the flowering time (Schulte auf'm Erley et al., 2007), as well as at harvest (Kamh et al., 2005; Ulas et al., 2012) (Figure II.7). The results of He et al., (2017) indicated that genotypes with high NUpE exhibited significantly larger ratios of root biomass to whole plant biomass than did those with low NUpE. Therefore, genotypic variation in N uptake mainly depended on N uptake rates per unit root weight (Yau and Thurling, 1987). Svečnjak and Rengel (2006a) reported no genotypic differences for root N-concentration during vegetative growth, highlighting N uptake differences were explained by variations in root dry weight.

Interestingly, Schulte auf'm Erley et al. (2007) found a significant genotypic difference for leaf senescence. Although genetic variation in N stem remobilization has been reported (Berry et al., 2010; Girondé et al., 2015), no correlation was found between the N content of the stems and the NUE or the NUtE at flowering (Stahl et al., 2015). Schulte auf'm Erley et al. (2011) showed that the genotypes with lower seed N content accumulated more dry matter in the seeds for a given amount of N accumulated, possibly explained by a higher photosynthetic rate of the pods or a higher efficiency of oil production (Bouchet et al., 2016). Moreover, He et al., (2017) pointed up the seed number per pod as a key trait supporting the observed genetic variation for NUtE at low and high-N conditions.

In a growth chamber study, [Koscielny and Gulden \(2012\)](#) found that root length in seedlings was indicative of NUE at harvest for eight winter oilseed rape genotypes. [Thomas et al. \(2016\)](#) identified seedling root traits (*i.e.* primary root length, lateral root length, and lateral root density) linked to seed biomass variation among 32 oilseed rape cultivars in six field experiments. [Wang et al. \(2017\)](#) found specific QTL for root morphology traits at early stages linked with NUE. However, the genotypic variability of root traits remains relatively unexplored in oilseed rape. As root growth and maintenance is costly in energy, the root architecture is a lever to optimize the balance between nitrogen absorption ability and metabolic costs ([Lynch, 2019](#)). From this perspective, increasing the root surface by improving fine roots density was considered as one possible strategy to increase NUE in other crops ([White et al., 2012](#)). Furthermore, the maintenance of higher green-leaf area (stay-green phenotype) along with high LAI and RUE might be related to the maintenance of N-uptake during the reproductive stage ([Diepenbrock, 2000](#); [Rathke et al., 2006](#)), and might be a source of genetic diversity for the enhancement of the NUE ([Bouchet et al., 2016](#)).

2.4.3. Genotype x Nitrogen interaction

Genotype x N-condition (G × N) interaction has been observed for NUE and its components in several crops such as wheat ([Górny et al., 2011](#); [Khan et al. 2017](#)) or maize ([Gallais and Hirel, 2004](#); [Apala Mafouasson et al., 2018](#)). Nevertheless, the study of the G x N interaction remains scarce in oilseed rape and has mainly been assessed at harvest ([Kessel et al., 2012](#); [Bouchet et al., 2014](#); [Nyikako et al., 2014](#); [He et al., 2017](#)). [He et al. \(2017\)](#) found G x N interaction on the shoot and root N-quantity and the root : shoot biomass ratio, but no significant G x N interaction was found on seed yield either using pots or under field conditions. In opposition to these results, [Nyikako et al., \(2014\)](#) revealed significant G x N interaction on seed yield, NUpE and NUtE, pointing out that selection for seed yield and NUE-components under high-N conditions would not result in genotypes generally suitable for low-N conditions. The G x N variability on NUtE has largely been studied in oilseed rape at canopy scale ([Berry et al., 2010](#); [Schulte auf'm Erley et al., 2011](#); [Kessel et al., 2012](#); [Nyikako et al., 2014](#); [He et al., 2017](#)) and at plant level ([Girondé et al., 2015](#); [Stahl et al., 2015](#); [He et al., 2017](#)). But only some studies have studied the G x N variability on the NUpE component and their impact on NUE and seed yield in other crops like wheat ([Melino et al., 2015](#)), maize ([Li et al., 2015](#)), and rice ([Rakotoson et al., 2017](#)).

However, breeding for NUE has been for a long time conducted under high-N conditions as the scope was generally to identify genotypes and traits that contribute to maximize the economic crop yield under common farming practices involving high N-input ([Van Bueren et al. 2017](#)). Over the last decades, breeding programs for NUE were conducted under both low- and high-N conditions as the scope was to identify genotypes and traits that contribute to improve NUE without compromising on yield under low N inputs. This is also illustrated by the modifications of variety registration protocols. Since 2004, registration protocols now include environmental concerns using VCUS criteria (Value for Cultivation, Use, and Sustainability) and potential varieties are experimented under reduced N practices (X-40 N units).

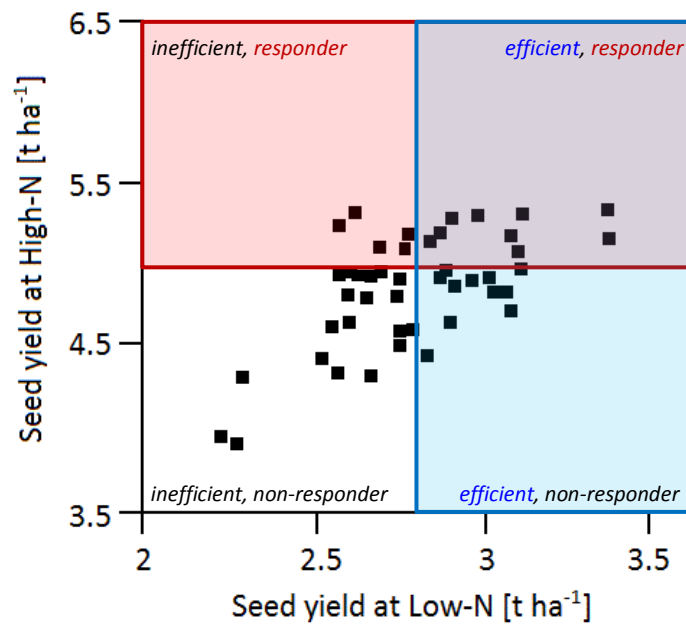


Figure II.8. Mean seed yields of 54 winter oilseed rape genotypes grown under two contrasting N-fertilization conditions (low-N, no fertilizer; high-N, 240 kg N ha⁻¹ of fertilizer). Data were acquired from field experiments conducted in three growing seasons at four locations in Germany. Rectangles divided the figure into four quadrants, showing the mean yield of the genotypes for each of the two N-conditions. Colors highlight the responding (red) and/or efficient genotypes (blue), according to the classification scheme of N efficiency and N response proposed by Gerloff (1977). The correlation for seed yield between low and high-N was $r = 0.60$. Adapted from Nyikako et al., (2014).

Several studies for different crops suggested that for identifying genetic NUE determinants and screening genotypes adapted to low-N conditions, a direct selection under low-N would be more effective than an indirect selection (Brancourt-Hulmel et al. 2005, Laperche et al. 2006, Hirel et al. 2007, Kessel et al., 2012; Stahl et al. 2017). However, genotypes that perform well under low-N can potentially show good responsiveness to high N availability as well (Han et al. 2015). Gerloff (1997) and Blair (1993) have referred to those genotypes as efficient responders using a classification based on yield response to two contrasting N-conditions (Figure I II.8). In oilseed rape, Nyikako et al., (2014), highlighted that seed yield correlation between low and high-N conditions was of only medium size ($r = 0.60^{**}$), suggesting the possibility of selecting genotypes with specific adaptation to low-N conditions.

Genotypes of winter oilseed rape presented contrasting NUE, due to differences in either nitrogen uptake efficiency (NUpE) or nitrogen utilization efficiency (NUE). One improvement lever might rely on the optimization of C and N resources acquisition during vegetative growth, which constitutes the N-available pool recycled to sustain the development of reproductive parts. This requires a thorough knowledge of the traits related to the development and growth of aerial and root organs, as well as a study of their genetic variability.

The absence of correlation between NUpE and NUE suggests that these two processes might be improved independently. However, research to improve NUE has been mainly carried out on NUE. Genetic variation exists for NUpE at both low and high-N conditions. Nevertheless, under low-N conditions, plant N uptake efficiency was the major lever sustaining genotypic differences in NUE at harvest.

Regarding traits, genotypic variability exists in oilseed rape for shoot and root traits, but it has mainly been assessed during the reproductive phase and/or at harvest. The observed genotypic variability in NUE at harvest has been linked with shoot (i.e. LAI) and root traits (i.e. root length and density) at an early growth stages (BBCH 16-18). Identifying early proxy traits for NUE would therefore enable a high throughput phenotyping of large panels of genotypes and growing conditions.

Regarding of N-condition, a genotype x N-condition (GxN) interaction has been observed in oilseed rape for seed yield and NUE, suggesting the possibility of selecting genotypes with specific adaptation to low-N conditions using a direct selection under low-N conditions. GxN variability of NUE has largely been studied in oilseed rape at canopy scale and at plant level. In contrast, some studies have focused on the NUpE component in different crops, but very few in oilseed rape.

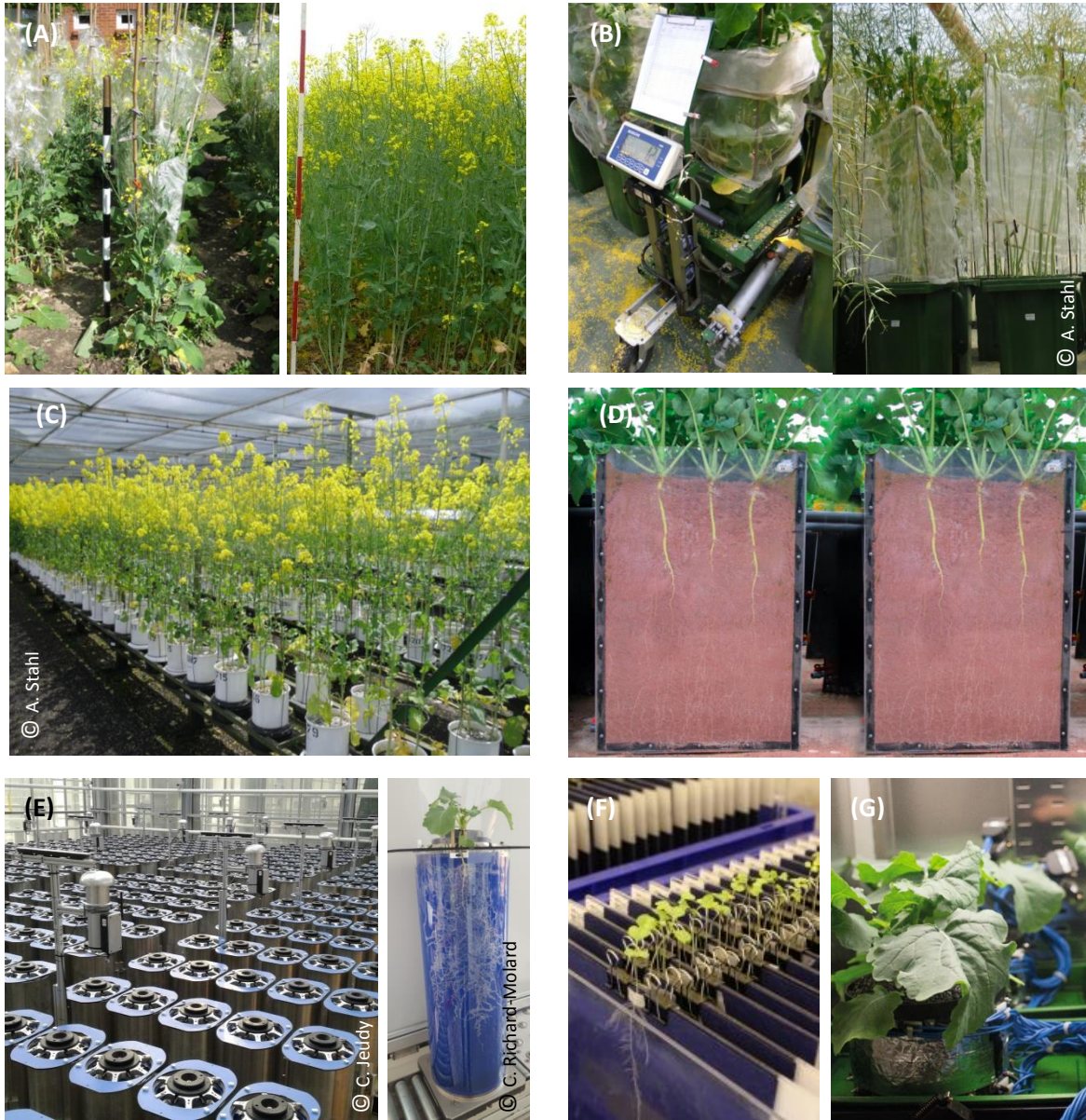


Figure II.9. Combined approaches for oilseed rape phenotyping A) in the field (Pommerrenig et al., 2018), B) under semi-controlled field-like conditions using containers (Hohmann et al., 2016), C) under controlled greenhouse conditions using Mitscherlich pots with several plants (Stahl et al., 2015), D) using rhizotrons (Yuan et al., 2016) or E) rhizotubes (Jeudy et al., 2016), and in a laboratory growing chamber using F) hydroponic pouches (Gioia et al., 2016) and G) electrical impedance tomography vessels (Corona-Lopez et al., 2019).

2.5. Phenotyping for N-use efficiency related traits in oilseed rape

It seems now clear that NUE genetic variability should be studied at both aboveground and below-ground plant levels (Svečnjak and Rengel, 2006a). Moreover, identifying early traits for phenotyping NUE directly under low N availability can be useful to accelerate breeding progress (Hohmann et al. 2016). Nevertheless, Balint and Rengel (2008) concluded that screening NUE oilseed rape for breeding purposes would require an assessment at maturity, as there was little consistency in NUE ranking between vegetative stage and maturity. In the same line, Bouchet et al. (2016) suggested that genotypes should be evaluated throughout the vegetative period until harvest to prevent the superiority of one trait being masked by the disadvantageous effects of another trait. Furthermore, Han et al. (2015) stressed the importance of developing accurate phenotyping and experimental designs and devices for evaluation complex traits such as NUE along the cycle and with a correct estimation of plant N-status, especially under low-N nutrition. Furthermore, the scope of improvement for NUE has been limited due to the inherent complexity of phenotyping roots (Garnett et al., 2009; Postma et al., 2014).

2.5.1. Phenotyping for NUpE-related traits requires adapted devices

One of the main difficulties in evaluating the influence of root traits and root architecture system on NUpE or directly on NUE and biomass is to access and/or remove the whole root system (including fine roots) from soil, when plants are grown under agronomic conditions (Guingo et al., 1998; Kondo et al., 2003). Thus, under field or field-like conditions, root sampling is usually limited to taproots (Malagoli et al. 2005; Sieling et al., 2017). However, there is not a standardized definition and threshold to identify fine roots (Laliberté, 2017). Fine roots have traditionally been classified based on an arbitrary diameter cutoff ≤ 2 mm diameter, and more recently some studies have assigned increasingly smaller diameter cutoffs (e.g. 1.0 or 0.5 mm) in an effort to explicitly emphasize more absorptive fine roots (reviewed in McCormack et al., 2015). Besides, this threshold have been reduced to ≤ 0.2 mm in other species (Picon-Cochard et al., 2012).

As reviewed by Adu et al. (2014) and Thomas et al. (2016), different root phenotyping methods can be directly used in the field, including root excavations (Oliveira et al., 2000; Trachsel et al., 2011; Bucksch et al., 2014; Arifuzzaman et al., 2019), soil-coring (Box and Ramsuer, 1993; Wasson et al., 2014), and the use of interfaces such as 'windows', trenches (Vepraskas and Hoyt, 1988) and mini-rhizotrons inserted into the soil (Dupuy et al., 2010). These techniques used to be highly time-consuming and laborious, destructive, prone to inaccuracy because small roots are lost during washing, and not adapted to screening large genetic populations (Zhu et al., 2011; Adu et al., 2014). Therefore, there are inherent difficulties in screening root in plants grown in the field (Laperche et al., 2006). Shovelomics is another method (Trachsel et al. 2011), which involves digging up the root system with shovel from the field, washing them and measuring the root traits with the help of a phenotyping board and/or imaging technology. It seems to be an adapted method to phenotype root traits in the field, such as root diameter, lateral root number and root angle. It has been recently used for phenotyping taproot traits in oilseed rape (Arifuzzaman et al., 2019), but it might not be adapted for fine root phenotyping.

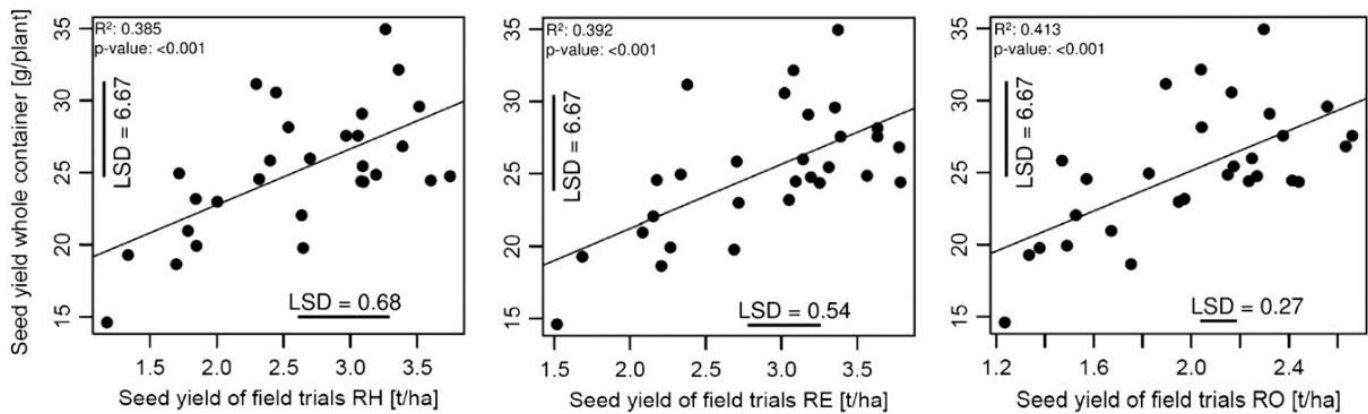


Figure II.10. Regression analyses for seed yield between the container plants and independent field trials in three locations in Germany under low-N inputs: Rauschholzhausen (RH), Reinshof (RE) and Rotenkirchen (RO). Comparing container yields against the average seed yield over all field locations, the authors suggested that containers were adapted to predict average field performance. Coefficients of determination for average single plant yield per container to average field performance were consistently under both N treatments (Low-N, $R^2=0.450$; High-N: $R^2 =0.432$). From Hohmann et al., (2016)

Alternative methods have been largely used to grow plants under controlled conditions. For example, oilseed rape has been grown in pots (Brunel-Muguet et al., 2015; Girondé et al., 2015; Stahl et al., 2015), rhizotrons (Jamont et al., 2013; Yuan et al., 2016), rhizotubes (Jeudy et al., 2016), gel-based mini-rhizotron (Kiran et al., 2019), aeroponics (Waisel, 2002), hydroponic culture systems (Rossato et al., 2001; Thomas et al., 2016; Weigand et al., 2017; Wang et al., 2017; Qin et al., 2019) and pouch system (Gioia et al., 2016; Shi et al., 2018). In contrast to field-based approaches, controlled growing conditions allow controlling and varying biotic and abiotic factors such as soil diversity, temperature, water and nutrient supply. These methods also facilitate non-destructive measurements of individual plants by using image analyses (Adu et al., 2014; Tomas et al., 2016). Other phenotyping technologies allowing to quantify root systems non-invasively and non-destructively are X-ray computed or electric tomography and magnetic or nuclear magnetic resonance imaging (reviewed by Paya et al., 2015 and Atkinson et al., 2019). The electrical impedance tomography has been successfully used in oilseed rape for root system characterization (Corona-Lopez et al., 2019).

However, Passioura (2012) and Hohmann et al. (2016) pointed out that results from controlled-environment rarely robust and generic enough to predict field performances on complex agronomic traits, such as NUE. Indeed, Dambreville et al. (2017) pointed up that the root restriction in small pots affects root growth and carbon partitioning. Studies assessing correlations of complex physiological parameters or yield-associated traits between pot and field experiments are rare (Poorter et al., 2012). In oilseed rape, He et al. (2017) evaluated the NUtE of 50 genotypes at harvest in pot and field experiments. The correlation between both experiments was very low ($r=0.34$) under the high-N condition and not significant under the low-N condition. Although the genotype ranking on NUtE was different between the pot and the field experiments, some genotypes performed consistently in both environments. The difficulty of comparing controlled experiments with field trials might rely on root growth constriction under controlled conditions, especially when measurements are done late in the growing cycle and for crops with a long lifecycle. Moreover, soil mechanical impedance (soil resistance) could be a constraint to generate a taproot system similar to that observed in the field (Whalley and Bengough, 2013). Passioura (2006) also postulated that the primary disadvantage pots is the limited interaction between plants. Crops in the field are always subject to neighbor interaction effects, whereas pot experiments typically avoid such effects (Hohmann et al., 2016).

To cope with pot size limitations, Hohmann et al. (2016), recently established a culture device with large containers (120L), hence using a soil volume that approximates field conditions and allows detailed phenotyping of small field-crop populations under the controlled environment of a greenhouse (Figure II.9). This system was successfully used to grown winter oilseed rape cultivars at field-like densities throughout the whole crop cycle, obtaining similar seed yields than those harvested in the field (Figure II.10). Although the authors conducted the container plants with two contrasted N-conditions (Low-N, 40 kg N ha⁻¹; High-N, 100 kg N ha⁻¹), no differences in biomass accumulation and seed yield were observed between N-conditions. It might have been resulted in higher net N mineralization of the soil in the containers, because of warmer soil temperatures.

[Bissuel-Belaygue et al. \(2015\)](#) developed a semi-controlled culture device, named PERISCOPE, for growing individual oilseed rape plants in a reconstructed canopy from emergence until seed maturity at harvest under field-like conditions. Their device allows to quantitatively access to each plant fraction, including fine roots at the plant level, and leads to phenotypes and seed yields similar to those of field-grown plants. It is also possible to dissociate contrasting N conditions and to combine measurements at the plant and crop scales. Thus, using this device, the authors were able to estimate NUE components dynamically at the crop and plant levels, at different phenological stages, under contrasted N- and soil conditions, through particularly accurate measurements including all shoot and root compartments.

2.5.2. A model-assisted phenotyping approach

Besides the experimental challenge for measuring NUE-related traits, especially fine root traits, the major complexity for assessing the genetic variability in NUE relies on the many underlying processes affecting biomass elaboration. NUE-related processes are often environment-dependent and show strong feedback and feedforward mechanisms during crop cycle and to N-availability ([Dresbøll et al. 2014](#); [Semenov et al., 2007](#)). As NUE is a trait resulting from dynamic process impacting biomass elaboration and oilseed rape has high growth plasticity, punctual NUE-related traits measurements along the crop cycle do not necessarily underlay the genetic variability for NUE in response to N-conditions. Furthermore, those traits might be highly auto-correlated or not indicative of the ultimate targeted process ([Ghanem et al., 2014](#)).

The use of crop models as a tool in oilseed rape researches has been proposed by several authors ([Diepenbrock, 2000](#); [Fourcaud et al., 2008](#); [Xu et al., 2011](#); [Robertson and Lilley, 2016](#)). Models for oilseed rape production are diversified in objectives and methodology. In some classic crop models, the plant canopy was divided into different layers, where the main processes of biomass production are computed for each layer separately and then integrated for the whole crop ([Tang et al., 2007, 2009](#); [da Luz et al., 2012](#)). Several models have incorporated the effects of mineral nutrition and their interactions with climate (air temperature, PAR, precipitation) and soil factors ([Petersen et al., 1995](#); [Habekotté, 1997](#); [Gabrielle et al., 1998a,b](#); [Jeuffroy et al., 2003](#); [Malagoli et al., 2005,2014](#); [Brunel-Muguet et al., 2015](#)). In most of them, the formalisms related to N fluxes in the soil-plant system take into account the process of leaf senescence as a main contributor of remobilized N compounds. [Böttcher et al. \(2020\)](#) recently published a winter oilseed rape dynamic crop growth model, which simulate dry matter production and partitioning, as well as N-uptake and N-distribution under optimal and water- and nitrogen-limited conditions during the whole crop cycle at the canopy scale. However, despite above ground dry matter was quite well simulated, the linear regression between measured and simulated root/shoot ratio gave a R^2 of 0.64; suggesting a potential lack on simulation of root growth. Moreover, variability on seed yield was not correctly estimated, probably due to variation in the harvest index, which was not included in the model.

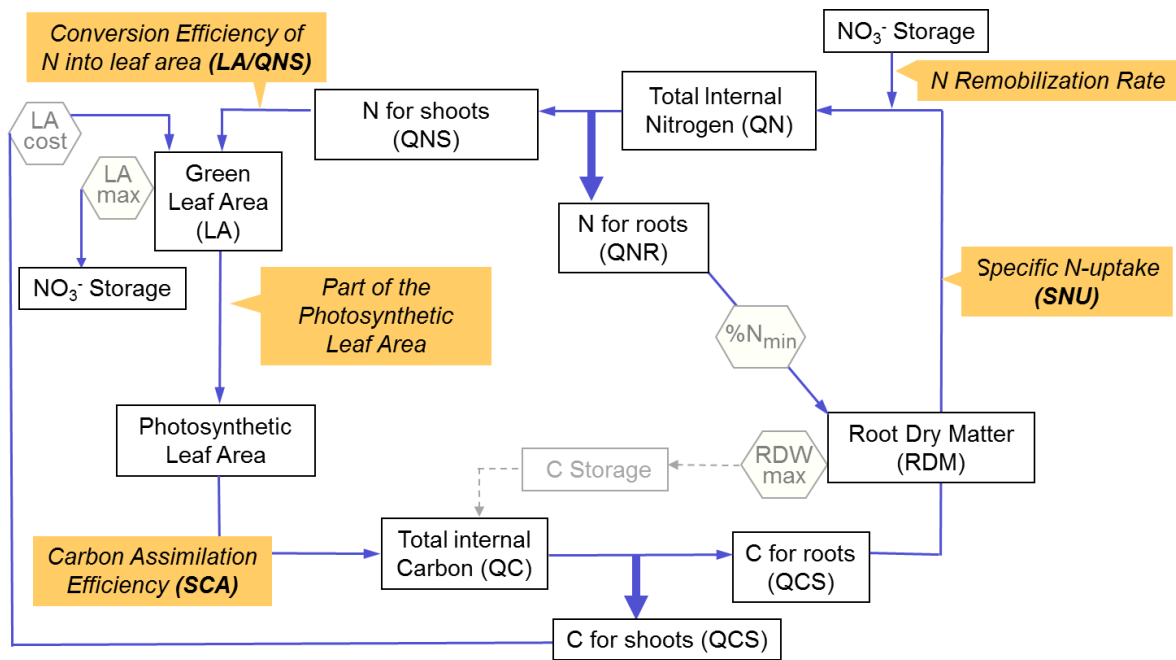


Figure II.11. Schematic diagram of the ARNICA's conceptual framework. This whole-plant structure-function model allows characterizing the N nutrition impact on the *Arabidopsis thaliana* whole plant functioning during the rosette growth period. It is based on interactions between N and C fluxes and offers a dynamic description of root system (considered as fine root biomass) and leaf growth (in terms of biomass and area). Lines represent the relationships between state variables and thickness the priority of the fluxes (given to shoots for C and to roots for N). Yellow boxes represent the efficiency parameters. From Richard-Molard et al. (2009).

At the plant level, model-assisted phenotyping approaches might help scientists to identify and hierarchize whole-plant parameters underlying the NUE genetic variation in response to N-conditions (Gu et al., 2014). Therefore, conceptual whole-plant functioning frameworks can be used as phenotyping tools to assess genotypic variations, by formalizing the observed relationships among whole-plant traits and dissecting integrative traits into simpler parameters (Hammer et al., 2006; Granier et al., 2014). Thus, the dynamic state variables characterizing the system are taken into account as well as the parameters controlling the relationships between state variables and factors influencing these variables (Wallach et al., 2013).

Estimating parameter values presents a major advantage, as by construction, parameters are supposed to reduce the residual environmental variance (Hirel et al., 2011) and exhibit a narrower variation than the associated state variables (Martre et al., 2015). Indeed, the state variables involved in NUE (*i.e.* biomasses, surfaces, and N-amounts) might exhibit wide variations in response to N-conditions, genotype, and developmental stage. However, efficiency parameters may present a narrower variation, which will help to rank the main processes underlying the NUE and highlight those that mainly cause the observed phenotypic variations (Laperche et al., 2006). Nevertheless, conceptual frameworks should be as simple as the nature of their objectives allow, not be overloaded with unnecessary details, and have minimum data requirements (Sinclair and Seligman, 1996). Moreover, plant models might be used to simulate traits that could not be measured on a large number of genotypes owing to resource or technical constraints (Chenu et al., 2017).

This plant-modeling approach has been successfully used to deal with the genotype x environment interaction and hierarchize the main physiological processes responsible for phenotypic differences in several species (*i.e.* in barley, Yin et al., 1999; maize, Reymond et al., 2003, Chenu et al., 2009; wheat, Laperche et al., 2006, Semenov, 2007, Bertin et al., 2010; sunflower, Lecoecur et al., 2011, Casadebaig et al., 2015; *Medicago truncatula*, Salon et al., 2009, Moreau et al., 2012b; and in *Arabidopsis thaliana*, Richard-Molard et al., 2009). These authors take into account the dynamics of plant growth and the relationships between C and N pathways, highlighting traits supporting the genotypic differences related to N-nutrition.

For example, the developed model by Richard-Molard et al. (2009), called ARNICA (Figure II.11), was developed to cope with plant's plasticity in response to N availability. It integrates processes at the whole plant scale and offers a dynamic description of root growth and leaf area expansion during vegetative growth. It combines integrative variables and parameters defined as efficiencies of plant CN functioning and morphogenesis. This simple compartmental model of C and N absorption and partitioning is compatible with a medium-throughput phenotyping of all 11 parameters included in the model.

In oilseed rape, [Groer et al., \(2007\)](#) developed a model considering the 3D structure and selected functions of the plant to compute the yield components under variable nitrogen fertilization by describing shoot organ formation and extension dynamics. Recently, [Tian et al. \(2017\)](#) developed a model based on the previous oilseed rape model proposed by [Groer et al. \(2007\)](#) and on the general sink-source-based functional–structural plant model developed by [Henke et al., 2016\)](#) to model the allometric relationships in leaves and predict the kinetics of carbon storage during autumn and winter.

Some more mechanistic models focused on the accurate description of photosynthesis rates, respiration, biomass production at the leaf and the shoot scales, including local climate variables as inputs ([Paul and Driscoll, 1997](#); [Müller and Diepenbrock, 2006](#)). Another plant model accounting for the interactions between source-sink relationships and architecture was developed by [Jullien et al. \(2007, 2011\)](#) to simulate the dynamics of shoot growth and development from sowing to seed maturity.

Despite numerous phenotyping devices available, NUE dynamic characterization remains tedious, and is still only possible for a limited number of genotypes. The characterization of plant traits related to NUpE, such as leaf senescence or N plant partitioning, has been more documented than NUpE because root phenotyping is still a bottleneck.

Phenotyping for NUpE-related traits requires adapted experimental devices allowing an accurate high-throughput and large-scale phenotyping of the whole plant system, including fine roots. Despite the inherent complexity of root phenotyping, its characterization is a major aim to further understand NUpE process and identify N-uptake related traits. However, at the canopy level and under field conditions, the acquisition of root traits for a large number of genotypes is still challenging. The identification of whole-plant traits under semi-controlled field-like conditions might be a good compromise, allowing access to the root systems of individual plants and the ability to correlate the identified traits to seed yield as observed in the field.

A model-assisted phenotyping approach might be relevant to identify and hierarchize whole-plant traits underlying the NUE genetic variation in response to N-conditions, as they often are environment-dependent and show strong feedback and feedforward mechanisms during the growing cycle and in response to N-availability. Such an approach might be useful to associate the dynamics of NUE-related state variables over the crop cycle with efficiency and allocation parameters, which are less dependent on the environment and on the compensation between processes. Assessing the genetic variation of parameters rather than state variables, could help identify and prioritize the traits determining the GxN variability of NUE from early stages in the growth of oilseed rape, characterized by high plasticity. However, a model suitable for analyzing NUE-related traits at the whole-plant scale is still lacking in oilseed rape.

Overview and highlights of Chapter II.

This chapter highlighted the need to clearly define NUE when discussing for its improvement or comparing results from different studies, as we accounted for nine definitions related to N-use efficiency. Furthermore, crop or plant NUE estimation deals with a lack on the estimation of the N-available in the soil during the crop cycle and the quantification of the total amount of N accumulated by the plants, including fallen leaves and fine roots. In this PhD work, we defined NUE as the ratio of whole plant dry matter (including fine roots and dead leaves) to N-available in the soil (including N fertilization and mineral N in the soil) (Chapter III).

From this chapter, we suggest a potential enhancement of oilseed rape performances in the context of lower N-inputs by improving N-uptake efficiency, but we also highlight that NUpE contribution to NUE was poorly documented, we investigated NUpE-related traits during vegetative growth, allowing a better understanding of the genotypic variability in NUE throughout the crop cycle under low-N conditions (Chapter III).

We emphasized that oilseed rape growth and development are complex, with the main periods of biomass accumulation and N uptake widely overlapping the period of N remobilization. Therefore, we pointed out the necessity of a better understanding of the dynamic on the C and N fluxes at the different growth phases and developmental stages to improve N-efficiencies in oilseed rape. Moreover, we highlighted that adapted phenotyping methods and approaches are required, especially regarding fine root phenotyping under field-like conditions. In this PhD work, we use three complementary experiments using a semi-controlled field-like device allowing access to the whole plant organs. Thus, we carried out multiple destructive samplings along the crop cycle, allowing characterizing biomass elaboration and N-uptake dynamics from leaves development to seed maturity (Chapter I, Figure I.2.).

Finally, we pointed out that a model-assisted phenotyping approach seems relevant to identify and hierarchize whole plant parameters underlying the NUE genetic variations to N-conditions, as they are interconnected and submitted to many feedbacks. However, models allowing a whole plant description over the entire crop cycle remains scarce in oilseed rape and there are no model describing N and C fluxes by considering shoot and root parts. Nevertheless, such an approach has been successfully used, for example, to analyze genotypic variability of whole-plant traits associated with N uptake and NUE in response to N deficiency in wheat (Laperche et al., 2006) and in *Arabidopsis thaliana* (Richard-Molard et al., 2009). Therefore, we proposed a conceptual framework to model oilseed rape C-N functioning at the whole-plant level, including shoot and root compartments, and used it to identify whole-plant traits explaining biomass elaboration in response to N conditions and its genotypic variation under low-N conditions (Chapter IV).

CHAPTER III.

Nitrogen Uptake Efficiency, mediated by fine root growth, early determines variations in Nitrogen Use Efficiency of rapeseed.

In the Chapter II we identified a scope of improvement for NUpE, which might drive the observed genotypic differences observed in NUE at harvest, especially under low-N conditions. However, we highlighted the current lack on phenotyping methods allowing characterizing and quantifying NUE-related processes, including dead leaves and fine roots. Indeed, dynamic analysis of the contributions of NUpE and NUtE to variations in NUE is still lacking in the literature.

The aim of this chapter is (i) to dynamically quantify the relative contributions of the NUpE vs. NUtE components to the temporal and genotypic variations of NUE throughout the winter oilseed rape crop cycle under two contrasting N conditions; and (ii) to unravel the processes underlying NUpE genotypic variability. Indeed, the NUpE component could become the main factor limiting NUE in the emerging context of reducing N inputs, yet its role in the determinism of NUE is still poorly documented.

We proceeded using two steps. First, we investigated a new variable related to NUE as a tool for analyzing NUE throughout the crop cycle. Second, we quantified the impact of NUpE, considered a dynamic process, on NUE and deciphered the relative contributions of NUpE and NUtE to NUE under two contrasting N conditions. Third, we focused on the sub-processes underlying NUpE (*i.e.*, Specific N-uptake and root growth) and characterized their genetic diversity in a set of genotypes representing the germplasm of winter oilseed rape.

This chapter has been written as a scientific paper, submitted to the Journal of Experimental Botany and currently under revision.

1. INTRODUCTION

Maintaining seed yield in a context of both increased climatic fluctuations and low nitrogen (N) inputs is a major issue for crop breeding and production. This is particularly relevant for winter oilseed rape, whose oil production represents ca. 15% of global vegetable oil production (FAOSTAT, 2017), but has depended greatly on N fertilizers over the past several decades (Berry and Spink, 2006). N fertilization is the main expense in the economic cost of the crop (Rothstein, 2007; Kant *et al.*, 2011), as well as a source of water pollution due to nitrate leaching (Di and Cameron, 2002) and air pollution due to N-derived greenhouse gas emissions (Sainju *et al.*, 2012). Breeding oilseed rape varieties adapted to low N inputs could therefore ensure a more sustainable and competitive agriculture. This current challenge relies on increasing N Use Efficiency (NUE).

NUE results from the product of two interacting components, N Uptake Efficiency (NUpE), corresponding to the proportion of available N in the soil taken up by the crop, and N Utilization Efficiency (NUtE), corresponding to the conversion of this absorbed N into seed yield, *i.e.* grain yield per unit of N taken up (Moll *et al.*, 1982). To date, most studies of oilseed rape NUE have focused on NUtE processes, assuming that the high N uptake capacities of oilseed rape (up to 100 kg N ha⁻¹ before flowering) was not the main process that limited NUE (Rossato *et al.*, 2001; He *et al.*, 2017). Thus, ecophysiological processes related to N accumulation in the plant throughout the crop cycle, such as NUpE, Specific N Uptake (SNU), and root growth, remain relatively unexplored, particularly during the vegetative phase. However, Lemaire *et al.* (2008) showed that the amount of N absorbed before flowering has a major influence on the leaf area index, a key trait determining plant biomass production and the final number of seeds (Bissuel-Belaygue *et al.*, 2011). The amount of N taken up before flowering could determine the yield potential (Colnenne *et al.*, 2002; Svecnjak and Rengel, 2006 a) which may depend on NUpE as well as on the architecture of the root system (Garnett *et al.*, 2009; Ulas *et al.*, 2012; He *et al.*, 2017) and its genotypic variability (Wang *et al.*, 2017). In addition, Richard-Molard *et al.* (2008) showed that the rate of N remobilization in response to N starvation in *Arabidopsis thaliana* is proportional to the amount of N previously accumulated, suggesting that remobilization efficiency during the reproductive phase may depend on NUpE during the vegetative phase.

Thus, improving the processes underlying NUpE during the vegetative phase should be particularly relevant for optimizing NUE, especially under conditions of low N input. This improvement relies on the genetic diversity available within the germplasm of winter oilseed rape, as well as on targeting relevant ecophysiological traits to be examined. Crop breeders have largely overlooked root traits as selection criteria to improve NUE, due to the difficulty in measuring them under field conditions (Robinson, 2004).

However, although few studies have considered the root compartment when characterizing genotypic variation in NUE in the field, some studies carried out on young plants in controlled conditions have highlighted the high genotypic variability in SNU and root architecture, traits that may influence NUpE (Laperche *et al.*, 2006; Wang *et al.*, 2017). No analysis of the genotypic variability of root architecture has yet been reported on mature plants, due to the persistence of phenotyping locks. Alternatively, a genotypic analysis of the fine-root growth, seen as a proxy of root architecture, could provide some clues of NUE determinism. Indeed, Wang *et al.* (2017) highlighted a strong correlation between root biomass and total root area on young plants of winter oilseed rape, and Louviaux *et al.* (2020) evidenced a positive correlation between primary root length early measured in hydroponics and seed yield measured in the field.

To accelerate breeding programs, screening oilseed rape varieties at early stages of development is attractive but remains challenging. Finding traits that can be phenotyped early in the crop cycle and quickly on many genotypes, and be relevant for explaining differences observed at harvest is difficult, as evidenced by conflicting results in the literature. On the one hand, Balint and Rengel (2008) showed little consistency between the NUE of 12 oilseed rape varieties measured at the vegetative and maturity stages. On the other hand, Koscielny and Gulden (2012), as well as Louviaux *et al.* (2020), found that seedling root length could be used as an early indicator of potential yield in winter oilseed rape and Wang *et al.* (2017) found QTL for root architecture traits that co-localized with QTL for NUE at the seedling stage.

However, an analysis of the dynamics of NUE components in response to N availability from sowing to harvest, highlighting the genetic variability in the underlying processes at the whole-plant scale, remains lacking. The objective of this study was to screen the main traits underlying the genotypic variation in NUE, including the fine-root compartment, with the aim to identify the main early contributors to NUE variations, particularly under low-N availability. We proposed a three-step strategy. First, we investigated a new variable related to NUE as a tool to early screen genotypic variability in NUE. Second, we analyzed the relative contributions of NUpE and NUtE to NUE throughout the growth cycle under two contrasting N conditions. Third, we focused on the sub-processes underlying NUpE (*i.e.* SNU and fine root growth) by distinguishing fine roots from tap roots, considering that they do not have an equivalent role in N uptake, and we characterized their genetic diversity in a set of seven genotypes representing the germplasm of winter oilseed rape.

Table 2. Sampling management: phenological stages and climatic conditions at each sampling date of the three experiments. Depending on the experiment, samples were taken at the beginning of rosette growth (BBCH 16-18), mid rosette development (BBCH 19), beginning of stem elongation after winter (BBCH 30-32), just before flower opening (BBCH 59) and end of the flowering period (BBCH 68-71). The final harvest (BBCH 84-89) was performed close to seed maturity and for all genotypes together, since the genotypes had similar phenology. The sum of growing degree-days (GDD) and the cumulative photosynthetically active radiation (PAR) from sowing are presented at each sampling date, for either the mean of all genotypes or for AVISO. The variation around each mean corresponds to the range of variation of these variables during the time required to harvest all plants for a given sampling date.

Experiment (code - site - year)	Sampling dates (day/month/year)	Days after sowing (DAS)	Phenological stages (BBCH scale)	Sum of growing degree-days (base 0°C)		Cumulative PAR (MJ m ⁻²)	
				genotype mean	Aviso	genotype mean	Aviso
LR15 Le Rheu 2014-2015	26/01/15 - 30/01/15	94 - 98	18	802 ± 26	798 ± 26	174 ± 6	173 ± 6
	23/03/15 - 29/03/15	150 - 156	31	1 148 ± 51	1 153 ± 51	377 ± 25	378 ± 26
	11/05/15 - 27/05/15	199 - 215	68	1 810 ± 204	1 810 ± 10	772 ± 146	769 ± 9
	23/06/15 - 29/06/15	242 - 248	84 (Harvest)	2 395 ± 117	2 384 ± 0	1161 ± 80	1154 ± 0
			Δ Harvest-maturity*	-355 ± 174	-366 ± 0	- 69 ± 6	-77 ± 0
GR15 Grignon 2014-2015	04/12/14 - 05/12/14	83 - 84	19		1 041 ± 4		325 ± 5
	05/02/15 - 06/02/15	146 - 147	30		1 321 ± 0		421 ± 3
	01/04/15 - 03/04/15	201 - 203	59		1 653 ± 17		649 ± 6
	04/05/15 - 06/05/15	234 - 236	71		2 052 ± 26		937 ± 19
	30/06/15 - 03/07/15	291 - 294	88 (Harvest)		2 972 ± 78		1597 ± 41
		Δ Harvest-maturity*		131 ± 0		69 ± 0	
GR18 Grignon 2017-2018	24/10/17 - 26/10/17	39 - 41	16	580 ± 27	565 ± 0	204 ± 6	200 ± 0
	12/12/17 - 14/12/17	88 - 90	19	905 ± 12	899 ± 0	302 ± 2	301 ± 0
	26/02/18 - 01/03/18	164 - 167	32	1 289 ± 0	1 289 ± 0	455 ± 13	449 ± 0
	10/04/18 - 13/04/18	207 - 210	59	1 616 ± 26	1 616 ± 7	655 ± 22	655 ± 5
	09/07/18	297	89 (Harvest)	3 090 ± 0	3 090 ± 0	1599 ± 0	1599 ± 0
		Δ Harvest-maturity*	242 ± 109	262 ± 0	154 ± 0	154 ± 0	

2. MATERIALS AND METHODS

2.1. Plant Material

Seven lines of winter oilseed rape (*Brassica napus* L.) were investigated in three experiments under two contrasting N conditions (Table 1). Genotypes were chosen to represent genetic diversity in winter oilseed rape, both in terms of release date (1980-2004) and type ('++' vs. '00' types, with high vs. low glucosinolate and erucic acid contents, respectively) (Table S1). They were selected from a panel of nearly 100 accessions, previously evaluated in the field (Bouchet *et al.*, 2016), for their contrasting seed yield and NUE response to N inputs. Attention was paid to compare genotypes with similar growth-cycle durations and dates of flowering (no more than 8 days between the two extreme genotypes) to minimize confounding effects between phenology and NUE processes. The genotype AVISO was assessed as a control in all experiments.

2.2. Experimental design

Three experiments (LR15, GR15, and GR18) were performed in two locations in France: LR15 was performed at Le Rheu (48°09'N, 1°76'W) during the 2014-2015 cropping season, while GR15 and GR18 were performed at Thiverval-Grignon (48°51'N, 1°58'E) during the 2014-2015 and 2017-2018 cropping seasons, respectively. Plants were grown on tubes 1 m high and 0.16 m diameter, with one plant per tube. Tubes were grouped into containers of 1m³ to reconstruct a canopy with a density of 35 plants m⁻². Plants were grown outdoors, under conditions similar to those of field experiments for rain, radiation and wind. In the LR15 experiment, each tube was filled with 26.8 kg of a soil/sand mixture (60:40, v/v), yielding a bulk density of 1400 kg m⁻³. In the GR15 and GR18 experiments, each tube was filled with 10.2 kg of an attapulgate/clay pebble mixture (50:50, v/v), yielding a bulk density of 520 kg m⁻³. In the containers, the space between tubes was filled with a sand/soil mixture to keep all root sections at the same temperature. In addition, to avoid edge effects, two rows of plants were planted in the sand/soil mixture surrounding the tubes. This culture device provided access to the shoot and root systems (including fine roots) of each plant from sowing to maturity (Bissuel-Belaygue *et al.*, 2015).

Six seeds of similar weight were sown in each tube from mid September to mid October. Seedlings were thinned twice during the first two weeks after emergence until only one medium-sized plant remained per tube. Pesticides were applied when necessary to control pests and diseases.

Experimental designs and sampling management are summarized in Table 1 and 2 respectively. In LR15, the experimental design consisted of a split-plot design with two N conditions as the main plot and six genotypes as sub-plots. In GR15, a single genotype (AVISO) was investigated under both N conditions, according to a complete randomized block design. In GR18, five genotypes were investigated under a single limiting N condition, except for AVISO, which was grown under both N conditions.

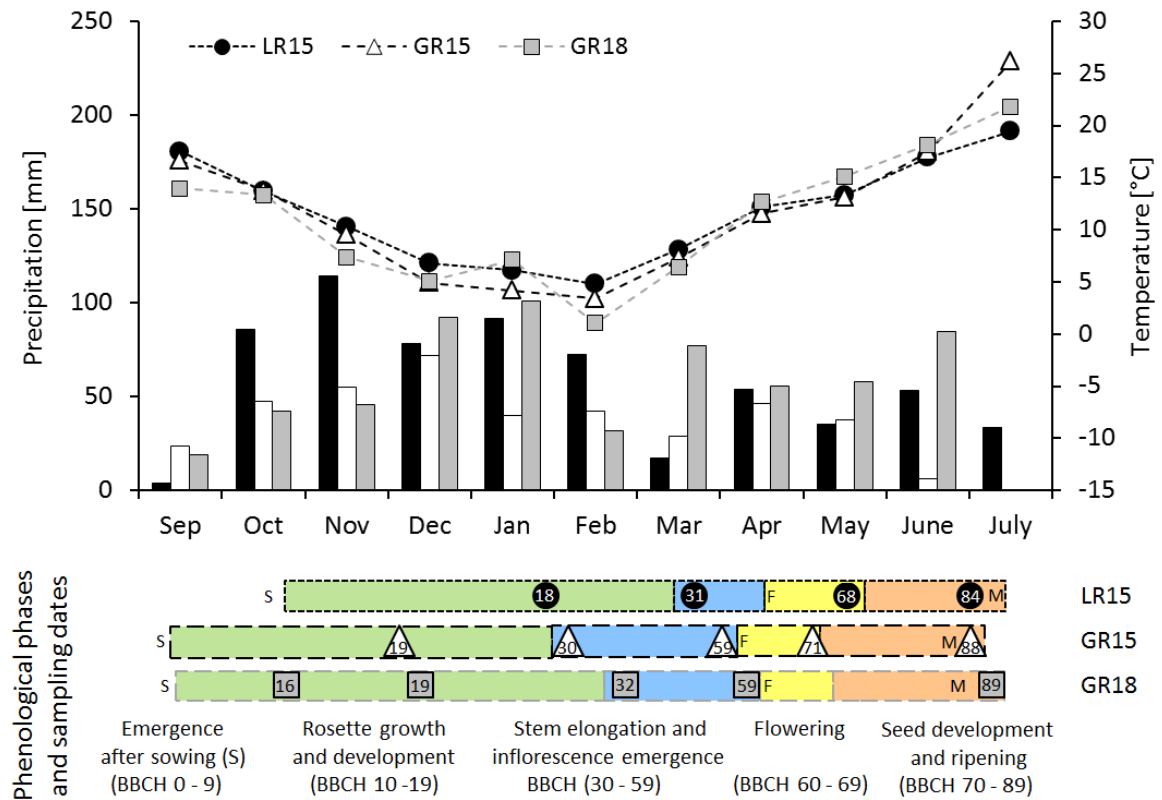


Figure 1. Total monthly precipitation (bars), mean monthly temperature (lines) and sampling dates and phenology of the AVISO genotype in the three experiments. Grayscale bars and symbols correspond to the three experimental sites × climate years (black circles, LR15; white triangles, GR15; gray squares, GR18). In the down panel, colors correspond to the 4 main phenological phases (green, rosette emergence, growth and development; blue, stem elongation and inflorescence emergence; yellow, flowering; orange, seed development and ripening), sampling dates are indicated by symbols, whereas numbers depict phenological stages according to the BBCH scale (Lancashire et al., 1991). Letters correspond to experimental management (S, sowing) or phenological markers (F, beginning of flowering; M, seed maturity).

2.3. Climate conditions

Daily mean air temperature ($^{\circ}\text{C}$), precipitation (mm), photosynthetically active radiation (PAR, MJ m^{-2}) and Penman evapotranspiration (mm) throughout the crop cycle were obtained from the INRA CLIMATIK platform (https://intranet.inrae.fr/climatik_v2/ClimatikGwt.html). Growing degree-days (GDD) were summed from sowing using a base temperature of 0°C (Dresbøll *et al.*, 2016). Because climate conditions differed among sites and years (Fig. 1), the duration of the growing cycle varied among experiments: 242-248, 291-294, and 297 days for LR15, GR15 and GR18, respectively. For the genotype AVISO in LR15, GR15, and GR18, thermal time between sowing and seed maturity were 2384, 2972, and 3090 GDD respectively, while cumulative PAR was 1154, 1597, and 1599 MJ m^{-2} , respectively (Table 2).

Five phenological phases were defined according to the BBCH scale (Lancashire *et al.*, 1991) to characterize the development of winter oilseed rape during the whole crop cycle: emergence (BBCH 0-9) rosette growth and development (BBCH 10-19), stem elongation and inflorescence emergence (BBCH 30-59), flowering (BBCH 60-69) and seed development and ripening (BBCH 70-89) (Fig. 1). Beginning of flowering (BBCH 60) was assumed to be reached when 10 % of primary inflorescence flowers had opened, and seed maturity was assumed to be reached at BBCH 69 + 940 GDD (Jullien *et al.*, 2011). The duration and climatic characteristics of the five phenological phases varied among experiments. LR15 had more precipitation and an overall deficit of cumulative PAR compared to GR15 or GR18 (Fig. 1, Table 1). GR18 had the most GDD during stem elongation and flowering period. Thus, plants harvested at the same BBCH stage may have accumulated slightly different GDD and PAR values.

2.4. Management of hydric and mineral conditions

Each tube was watered to the soil's water holding capacity at the beginning and throughout the experiment with a modified Hoagland solution that provided no N [3 mM KCl, 1 mM MgSO_4 , 0.5 mM KH_2PO_4 , 2.5 mM CaCl_2 , 27 μM Fe-EDDHA, 30 μM H_3BO_3 , 10 μM MnSO_4 , 1 μM ZnSO_4 , 0.1 μM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, 0.5 μM CuSO_4 , 0.5 μM CoCl_2]. During experiments, cumulative precipitation (mm) and Penman evapotranspiration (mm) were used to estimate the soil water balance and manage Hoagland solution supplies to maintain soil moisture above 85% of field capacity, thus avoiding water stress and nutrient loss through leaching.

N was provided by a solution of KNO_3 and $\text{Ca}(\text{NO}_3)_2$ (1:1 valence) mixed with the modified Hoagland solution and supplied every 200 GDD from emergence (BBCH 09) to harvest (BBCH 84-89), resulting in 13 to 14 applications during the growth cycle. The amounts of N applied per tube were calculated to generate two contrasting N conditions from emergence: low N, with a limiting cumulative N supply of 0.22-0.26 g per plant (equivalent to 25-29 kg N ha^{-1} in the field), and high N, with a non-limiting cumulative N supply of 1.47-1.79 g per plant (equivalent to 165-200 kg N ha^{-1}) (Table 1). In addition to N applications, the mineral N initially present in the substrate was taken into account to quantify the mineral N available in the soil (QN soil). Homogeneous samples of substrate (50 g) were collected at three key stages common to all experiments (BBCH 16-18, BCH 30-32, and BBCH 59) at three depths (0-30, 30-60, and 60-90 cm) in several tubes to quantify water, NO_3^- and NH_4^+ contents using the Kjeldahl (1883) method. The N Nutrition Index (NNI) for C_3 plants (Lemaire and Gastal, 1997) was used to quantify the plant N status generated by each N condition (Table 1).

Table 3. Effects of N condition and genotype on variation in the dry-matter-based N Use Efficiency (NUE_DM) and its components during the crop cycle in the three experiments. Six key phenological stages were targeted: BBCH 16-18 (beginning of rosette growth), BBCH 19 (mid rosette development), BBCH 30-32 (beginning of stem elongation), BBCH 59 (just before flower opening), BBCH 68-71 (end of flowering), and BBCH 84-89 (seed maturity). For each stage, values of NUE_DM, NU_pE, NU_tE, and NUE_Seed represent the mean of all genotypes cultivated in the same experiment and of AVISO alone. The significance of genotype (G) (GR18 and LR15 only), nitrogen condition (N) (GR15 and LR 15 only), and genotype × N (G×N) interaction (LR15 only) effects was assessed for each experiment separately. Effects are expressed as a percentage of total variation (%var or, for LR15, variance decomposition). Significance codes: *** *P*-value < 0.001, ** *P*-value < 0.01, * *P*-value < 0.05. NS: non-significant.

Sampling stage		GR18 (Grignon 2017-2018)				GR15 (Grignon 2014-2015)				LR15 (Le Rheu 2014-2015)									
		Low-N genotype mean		%var		Low-N	High-N	%var		Low-N genotype mean		High-N genotype mean		Variance decomposition					
Trait	Unit	AVISO		G	AVISO	AVISO	N		AVISO	AVISO	AVISO	AVISO	G	N	G×N				
BBCH 16-18																			
NUE_DM	g gN ⁻¹	1.39	1.28	57%	***	–	–	–	2.08	3.06	1.18	1.36	23%	***	31%	***	14%	**	
NU _p E	gN gN ⁻¹	0.07	0.07	53%	***	–	–	–	0.08	0.12	0.05	0.06	23%	***	30%	***	13%	**	
NU _t E	g gN ⁻¹	19.99	19.80	6%	ns	–	–	–	24.50	25.70	24.40	23.90	45%	***	<1%	ns	9%	ns	
BBCH 19																			
NUE_DM	g gN ⁻¹	11.29	10.80	57%	***	19.57	12.23	55%	**	–	–	–	–	–	–	–	–	–	
NU _p E	gN gN ⁻¹	0.39	0.37	62%	***	0.59	0.47	22%	ns	–	–	–	–	–	–	–	–	–	
NU _t E	g gN ⁻¹	29.02	29.40	35%	*	33.04	25.63	89%	***	–	–	–	–	–	–	–	–	–	
BBCH 30-32																			
NUE_DM	g gN ⁻¹	23.62	24.30	15%	ns	28.45	12.73	86%	***	12.40	17.15	6.90	7.24	18%	***	48%	***	17%	***
NU _p E	gN gN ⁻¹	0.68	0.71	6%	ns	0.68	0.42	65%	**	0.33	0.44	0.31	0.27	20%	***	33%	*	17%	**
NU _t E	g gN ⁻¹	34.86	34.10	64%	***	39.04	30.07	68%	***	37.20	38.70	22.30	21.30	5%	***	87%	***	4%	***
BBCH 59																			
NUE_DM	g gN ⁻¹	36.02	42	68%	***	38.88	30.55	42%	*	–	–	–	–	–	–	–	–	–	
NU _p E	gN gN ⁻¹	0.74	0.78	22%	ns	0.84	0.74	33%	ns	–	–	–	–	–	–	–	–	–	
NU _t E	g gN ⁻¹	48.54	54.1	72%	***	46.39	40.76	40%	*	–	–	–	–	–	–	–	–	–	
BBCH 68-71																			
NUE_DM	g gN ⁻¹	–	–	–	–	70.83	33.74	99%	***	61.30	62.40	41.80	46.20	10%	ns	44%	***	7%	ns
NU _p E	gN gN ⁻¹	–	–	–	–	0.83	0.74	60%	**	0.75	0.79	0.68	0.64	13%	ns	7%	*	9%	ns
NU _t E	g gN ⁻¹	–	–	–	–	85.42	46.00	98%	***	82.80	82.50	62.00	72.20	32%	***	50%	***	4%	*
BBCH 84-89																			
NUE_DM	g gN ⁻¹	59.20	66.50	55%	***	72.03	27.96	98%	***	68.20	70.40	42.03	43.05	11%	**	62%	***	3%	ns
NU _p E	gN gN ⁻¹	0.70	0.74	36%	*	0.75	0.51	94%	***	0.85	0.85	0.63	0.66	7%	ns	57%	***	5%	ns
NU _t E	g gN ⁻¹	84.25	86.70	52%	**	95.84	54.75	97%	***	79.70	82.90	67.10	65.10	30%	***	43%	***	4%	ns
NUE_Seed	g gN ⁻¹	14.72	15.92	62%	***	18.33	7.02	98%	***	16.20	14.20	10.80	10.40	16%	**	42%	***	9%	*
Seed Yield	t ha ⁻¹	2.48	2.70	61%	***	3.28	4.46	76%	**	2.29	2.02	6.29	6.03	3%	*	85%	***	2%	ns

2.5. Sampling and measurements

For each genotype and N condition, five to eight replicates were harvested at multiple phenological stages throughout the crop cycle (Fig. 1, Table 2). In the LR15 experiment five replicates per treatment (*i.e.* genotype × N combination) were sampled four times (including harvest). In GR15 and GR18 experiments, six and seven to eight replicates respectively were sampled five times (including harvest).

The entire root system was carefully collected, paying special attention to recover all fine roots from the substrate. In addition, all senescing and dead leaves were counted and collected throughout the experiment. At each sampling date, harvested plants were divided into fractions: tap roots, fine roots, leaves (green, senescing and dead), main stem, branch stems, and pods (including immature seeds or, when dehiscent, seeds and pod walls). The dry matter (DM) of each plant fraction was weighed after lyophilization or oven drying at 70°C. Samples were then ground to a fine powder and analyzed for carbon (C) and N content according to the Dumas combustion method (Buckee 1994), using an automated CN analyser (Vario MICRO Cube, Elementar France, Lyon, France). In the LR15 experiment, seed N content was estimated using near-infrared reflectance spectroscopy (MPA, Multi Purpose FT-NIR Analyser, Bruker Optics, Ettlingen, Germany). Seed yield was calculated at the crop scale and expressed in t ha⁻¹, considering a plant density of 35 plants m⁻². The mean seed yield of AVISO was 6.03, 4.46 and 7.56 t ha⁻¹ under the high-N condition and 2.02, 3.28, and 2.70 t ha⁻¹ under the low-N condition in LR15, GR15, and GR18 respectively (Table 3), which matched with seed yields already reported in the field (Stahl *et al.*, 2017, Corlouer *et al.*, 2019).

2.6. Variables calculated

Depending on the variable considered, data were expressed either per plant fraction, per plant (all fractions), for shoots (aboveground fractions), or for roots (belowground fractions).

At each sampling date, a DM-based NUE (NUE_DM, g gN⁻¹) was calculated at the plant scale as the ratio of whole-plant DM (DM, g plant⁻¹, including tap and fine roots and senescing and dead leaves) to the quantity of soil mineral N available for plant growth (Soil_QN, gN plant⁻¹), the latter being calculated by summing mineral N initially available from the soil and N applications [Eq. 1]:

$$NUE_DM = \frac{Root_DM + Shoot_DM}{Soil_QN} \quad [Eq. 1]$$

At harvest, NUE_DM was compared to NUE_Seed (g gN⁻¹), calculated as the ratio of seed DM (g plant⁻¹) to Soil_QN from sowing to harvest (gN plant⁻¹).

NUpE (gN gN⁻¹) was calculated as the ratio of the QN accumulated in the whole plant to Soil_QN [Eq. 2]:

$$NUpE = \frac{Root_QN + Shoot_QN}{Soil_QN} \quad [Eq. 2]$$

NUtE (g gN⁻¹) was calculated as the ratio of whole-plant DM to the QN accumulated in the whole plant [Eq. 3]:

$$NUtE = \frac{Root_DM + Shoot_DM}{Root_QN + Shoot_QN} \quad [Eq. 3]$$

SNU (gN g⁻¹) was calculated as QN accumulated in the whole plant per g of cumulative fine-root biomass [Eq. 4]:

$$SNU = \frac{Root_QN + Shoot_QN}{Cumulative\ Fine\ Root\ DM} \quad [Eq. 4]$$

Cumulative fine-root biomass was calculated from destructive measurements of fine-root DM. Two logistic functions were used to fit the dynamics of fine-root biomass accumulation under each N condition, one from emergence to winter and a second from winter to harvest, with the following logistic equation [Eq. 5]:

$$f(t) = c / (1 + b \times e^{-at}) \quad [Eq. 5]$$

Parameters a, b and c were adjusted to minimize the sum of squares deviation, using the Generalized Reduced Gradient method for nonlinear optimization (Lasdon *et al.* 1974). The integral of the fitted curve, representing cumulative fine-root biomass, was approximated using a Riemann's sum.

2.7. Component-contribution analysis

The contribution of the components NUpE and NUtE to the variation in NUE_DM was calculated at each sampling date and analyzed under each N condition, as developed by Moll *et al.* (1982) and used for oilseed rape by Kessel *et al.* (2012) and Nyikako *et al.* (2014). Contribution analysis consists of linearizing the multiplicative relationship between NUE_DM, NUpE and NUtE by log-transforming it [Eq. 6]:

$$\log(NUE_DM)_i = \log(NUpE)_i + \log(NUtE)_i \quad [Eq. 6]$$

The relative contribution of NUpE and NUtE (component traits) to the variation in NUE_DM (resultant trait) is then calculated according to Eq. 7a and 7b, respectively :

$$NUpE\ relative\ contribution = \frac{\sum \log(NUpE)_i \times \log(NUE_DM)_i}{\sum \log(NUE_DM)_i^2} \quad [Eq. 7a]$$

$$NUtE\ relative\ contribution = \frac{\sum \log(NUtE)_i \times \log(NUE_DM)_i}{\sum \log(NUE_DM)_i^2} \quad [Eq. 7b]$$

2.8. Statistical analysis

Statistical analyses were performed and plots were generated using R software v. 3.4.2 (R Core Team, 2013). Pearson's correlation coefficients (r) were calculated from the means of all genotypes and Holm's correction was applied for the evaluation of correlation significance. Parameters of nonlinear models (*i.e.* logistic curves and exponentials) were adjusted using the nls() function (Bates and Watts, 1988; Bates and Chambers, 1992). Linear regression models were fitted with the lm() function. Type II analyses of variance (ANOVA) were performed using the 'car' package of R, and Tukey's post-hoc procedure was used to compare means. ANOVA assumptions were tested using the Shapiro-Wilk and Levene's tests. Hotelling's (1931) T-squared distribution test was used to test the multiple parameters of the nonlinear models. Statistical significance was estimated at $\alpha = 5\%$.

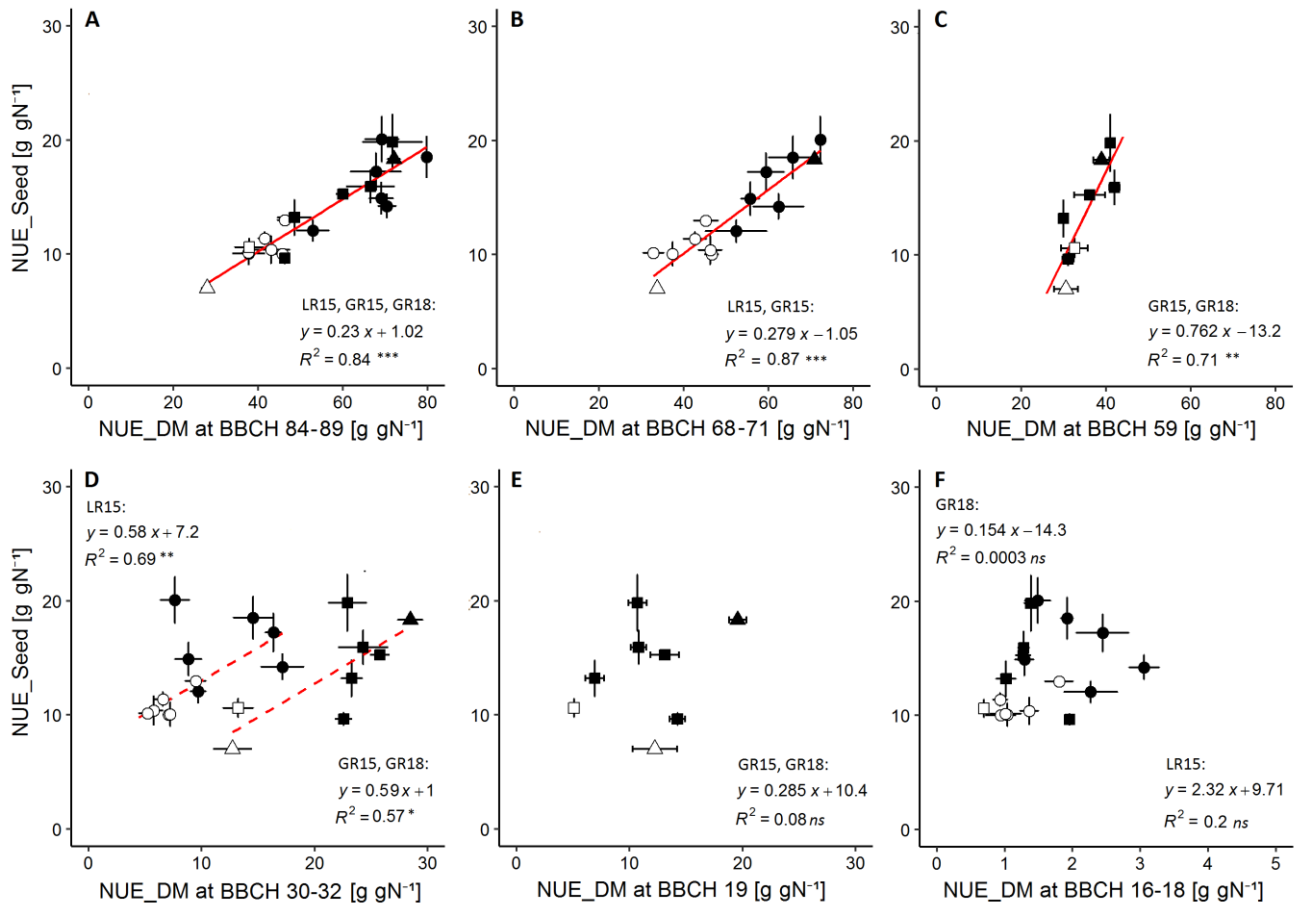


Figure 2. Relationships between NUE_Seed measured at seed maturity (BBCH 84-89) and NUE_DM throughout the crop cycle. NUE_DM was measured at A) seed maturity (BBCH 84-89), B) end of flowering (BBCH 68-71), C) just before flower opening (BBCH 59), D) beginning of stem elongation after winter (BBCH 30-32), E) mid rosette development (BBCH 19) and F) early rosette development. Regressions were performed by pooling data for all sites x years x N conditions when no significant differences on relationships parameters were found by separately comparing site, year or N condition effects. Solid lines indicate significant regressions ($p < 0.05$) validated both for each N condition separately as well as by pooling N conditions. Dashed lines indicate significant regression (P -value < 0.05) valid only when grouping N conditions. Open symbols correspond to the high-N condition, while filled symbols correspond to the low-N condition, with circles for LR15, triangles for GR15, and squares for GR18. Error bars depict standard errors of the mean.

3. RESULTS

3.1. Relating NUE_Seed to NUE_DM at seed maturity and at earlier stages

We aimed at validating NUE_DM as a new variable reliably reflecting NUE_Seed variations at seed maturity. NUE_Seed values of the AVISO genotype ranged from 7.02 to 10.40 g gN⁻¹ under the high-N condition and from 14.20 to 18.33 g gN⁻¹ under the low-N condition (Table 3). At seed maturity (BBCH 84-89), NUE_DM of AVISO ranged from 27.96 to 43.05 g gN⁻¹ under the high-N condition and from 66.50 to 72.03 g gN⁻¹ under the low-N condition (Table 3). Interestingly, by pooling data from all sites, years, genotypes, and N conditions, we identified a strong and unique linear relationship between NUE_Seed and NUE_DM calculated at harvest ($R^2 = 0.84$; P -value = 1×10^{-8}) (Fig. 2A), highlighting that NUE_DM at seed maturity was closely related to NUE_Seed, regardless of genotype, climatic condition, or N condition.

We investigated this relationship throughout the crop cycle to determine how early NUE_DM became a good proxy for NUE_Seed. NUE_DM increased continuously during the crop cycle, due to the continuous increase in the total plant biomass (since dead leaves were included) relative to the quantity of N available in the soil (Table 3). In addition, NUE_DM was always 1.3-2.6 fold higher under the low-N condition than the high-N condition (P -value < 0.001). We tested the relationship between NUE_Seed (calculated at seed maturity) and NUE_DM calculated at five earlier phenological stages: end of flowering (BBCH 68-71), just before flower opening (BBCH 59), beginning of stem elongation (BBCH 30-32), and mid- and early rosette growth (BBCH 19 and BBCH 16-18, respectively) (Figs. 2B to 2F). Regardless of N condition and genotype, NUE_Seed had a strong relationship with NUE_DM at BBCH 68-71 ($R^2 = 0.87$; P -value = 1.4×10^{-6}) and BBCH 59 ($R^2 = 0.71$; P -value = 0.009) (Figs. 2B, 2C). Moreover, we were able to evidence that the relationship was common to both experimental sites at BBCH 69-71, and to both experimental years at BBCH 59. At BBCH 30-32, site-specific relationships were observed, due to differences in intercept but with the same slope (Fig. 2D). The relationships observed at BBCH 30-32 were mainly driven by N conditions, as they became not significant when considering genotypes in a single N condition. At BBCH 19 and BBCH 16-18, the relationships became non-significant (Figs. 2E, 2F). Thus, NUE_DM measured as early as BBCH 59 could be used as a robust proxy trait to represent NUE_Seed of genotypes at seed maturity in all N conditions. At BBCH 30-32, the proxy is still valid to discriminate N conditions but not accurate enough to discriminate genotypes.

The relationships between NUE_DM at seed maturity and that earlier in the growth cycle were similar ($R^2 = 0.88$, P -value = 7×10^{-7} and $R^2 = 0.75$, P -value = 5×10^{-3} at BBCH 68-71 and BBCH 59 respectively ; $R^2 = 0.71$, P -value = 1×10^{-3} and $R^2 = 0.73$, P -value = 7×10^{-3} at BBCH 30-32 for LR15 and GR15+GR18 respectively) (data not shown), indicating that NUE_DM may also be a relevant variable for dynamically clarifying NUE shaping in various genotypes as early as BBCH 59 and in response to N supply as early as BBCH 30-32.

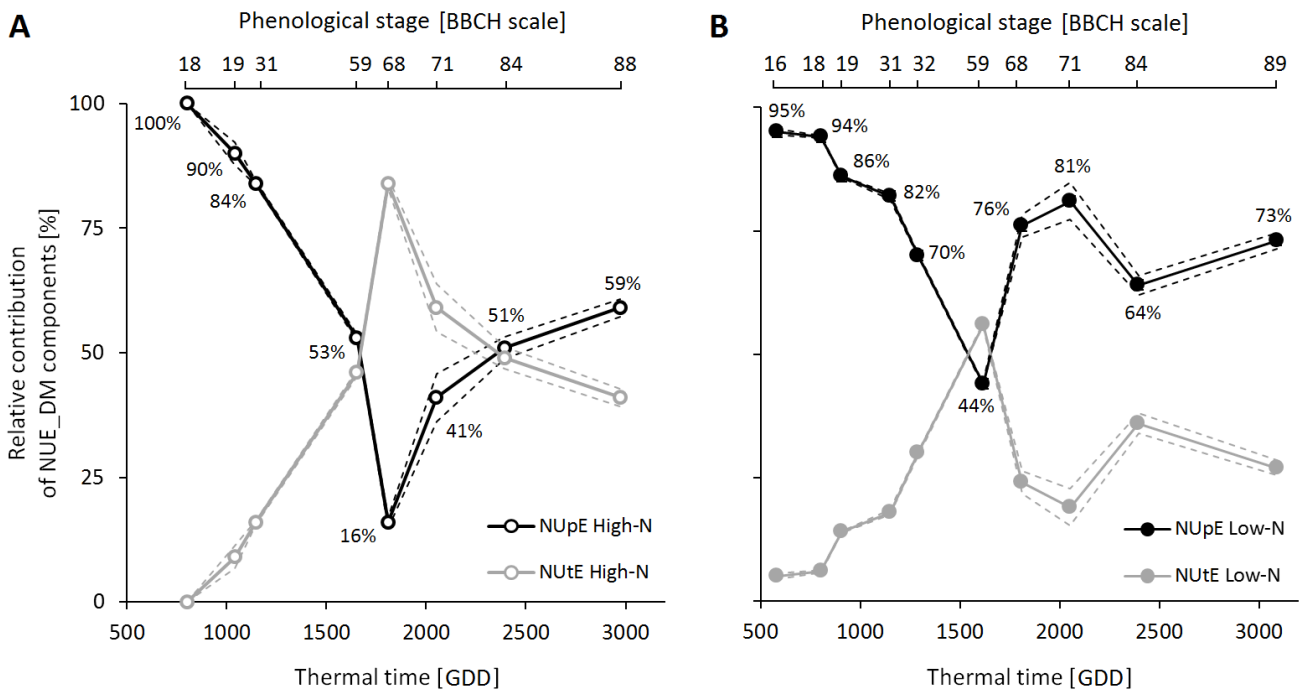


Figure 3. Contribution dynamics of N Uptake Efficiency (NUpE) and N Utilization Efficiency (NUTE) to variations in dry-matter-based N Use Efficiency (NUE_DM) throughout the crop cycle under high-N (A) and low-N (B) conditions. Relative contributions were calculated by pooling data for all genotypes from LR15, GR15 and GR18 experiments. Black and gray symbols correspond to $\log(\text{NUpE})$ and $\log(\text{NUTE})$, respectively. Dashed lines depict 95% confidence intervals. The percentage values correspond to the NUpE contributions to NUE_DM.

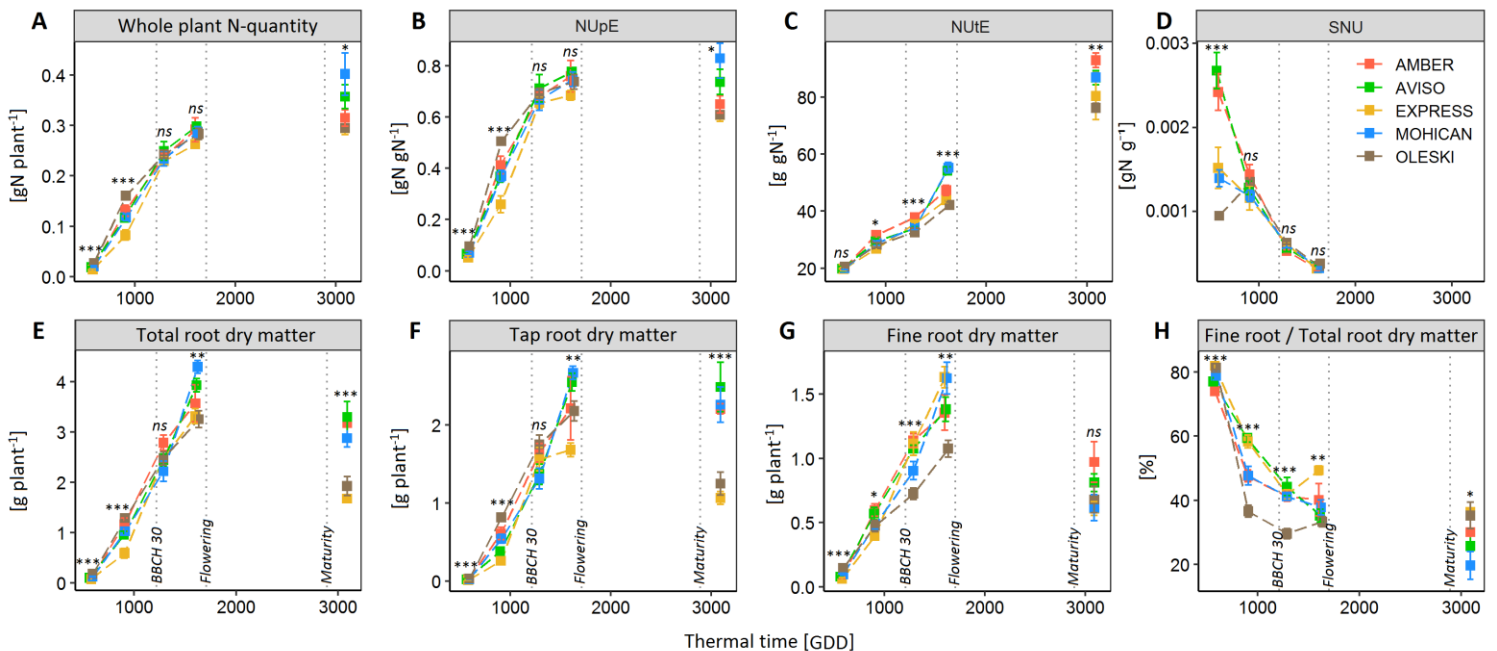


Figure 4. Dynamics of N accumulation in the whole plant (A), N Uptake Efficiency (NUpE) (B), N Utilization efficiency (C), Specific Nitrogen Uptake (SNU) (D), total-root (E), tap-root (F) and fine-root (G) dry matter, and fine- to total root ratio (H) during the crop cycle for five genotypes (AMBER, AVISO, EXPRESS, MOHICAN, OLESKI) grown under the low-N condition (GR18 experiment). Each genotype is represented by a different color. Vertical dotted lines depict the end of winter (BBCH 30), the beginning of flowering (BBCH 60) and the seed maturity (BBCH 84). Error bars indicate standard errors of the mean. Significance codes: *** P -value < 0.001, ** P -value < 0.01, * P -value < 0.05, ns: non-significant.

3.2. Dynamic contribution of NUpE and NUtE to NUE_DM

Dynamic analysis of the two NUE components at six sampling dates during the crop cycle indicated that NUpE and NUtE, like NUE_DM, were lower under the high-N condition than the low-N condition (Table 3). Three contrasting phases emerged from the dynamic analysis of relative contributions of NUpE and NUtE to NUE_DM throughout the crop cycle (Fig. 3). During the vegetative phase (BBCH 16-18 to 59), the contribution of the NUpE was first predominant (95-100%) and then decreased steadily up to flowering, but still accounted for 44-53%, regardless of the N condition. During the flowering period (BBCH 60-69), contrasted patterns distinguished according to N conditions. In the high-N condition, the relative contributions of NUpE and NUtE inverted, with NUtE becoming the main NUE_DM component (84%) and the NUpE's contribution decreasing sharply to 16%. In contrast, in the low-N condition, the contribution of NUpE increased strongly again to reach 76%. Finally, during seed development and ripening (BBCH 70-89), NUpE contribution leveled off at 64-81% under the low-N condition, and 41-59% under the high-N condition, indicating that NUpE still played a significant contribution during this phase, especially when N supply was limiting.

3.3. Genotypic variation in NUE_DM and its components

A high and significant genotypic variability in NUE_DM during the vegetative phase was identified using the LR15 and GR18 datasets, since the genotype effect explained up to 68% of the overall variation in NUE_DM, depending on the site and climate year (Table 3). In contrast, the genotype effect decreased during the reproductive phase, suggesting that the NUE_DM genotypic variability was determined mainly during the vegetative phase. The same dynamics was observed for NUpE, but with a more drastic reduction in genotypic variability after flowering than the one observed for NUE_DM. Indeed, genotype effects were significant only up to the beginning of stem elongation (BBCH 31) (Table 3). NUpE increased up to the end of the vegetative phase (BBCH 59), and then leveled off (AVISO and MOHICAN) or slightly decreased (AMBER, EXPRESS, and OLESKI) during the reproductive phase (Fig. 4). At BBCH 59, AVISO and MOHICAN had the highest NUpE (0.78 and 0.75 gN gN⁻¹, respectively), whereas EXPRESS had the lowest (0.68 gN gN⁻¹) (Table S2). Even if the differences were not significant, this tendency may explain the significant differences observed in NUpE at seed maturity.

On the other hand, NUtE significantly differed between genotypes from BBCH 18 onwards (Table 3) and slight differences between AMBER and EXPRESS appeared at BBCH 19 (Table S2, Fig. 4). From BBCH 32 to harvest, NUtE increased for all genotypes and three groups of genotypes can be distinguished: AVISO and MOHICAN which had high NUtE values at BBCH 59 and harvest, EXPRESS and OLESKI which had the lowest values of NUtE both at BBCH 59 and harvest, and finally AMBER which had one of the lowest values of NUtE at BBCH 59 (not significantly different from EXPRESS and OLESKI) but the highest NUtE value at harvest (not significantly different from AVISO and MOHICAN). In any case, the percentage of NUtE variance explained by the genotypic effect increased from BBCH 59 onwards, far exceeding that of NUpE.

Table 4. Regression analysis between dry-matter-based N use efficiency (NUE_DM) and N uptake Efficiency (NUpE) or N Utilization Efficiency (NUtE) under low-N and high-N conditions during the crop cycle. Six key phenological stages were targeted: BBCH 16-18 (early rosette development), BBCH 19 (mid rosette development), BBCH 30-32 (beginning of stem elongation), BBCH 59 (just before flower opening), BBCH 68-71 (end of flowering), and seed maturity (BBCH 84-89). For each sampling date, analyses were performed on the mean values of each of the winter oilseed rape genotypes grown under low-N conditions at Grignon (GR18) and under low-N and high-N conditions at Le Rheu (LR15). The table shows Pearson’s correlation coefficients (r) and significance of each regression, with *** *P*-value < 0.001, ** *P*-value < 0.01, * *P*-value < 0.05. Holm’s correction was applied for the evaluation of correlation significance. NS: non-significant.

	LR15		LR15		GR18	
	High-N		Low-N		Low-N	
	NUpE	NUtE	NUpE	NUtE	NUpE	NUtE
BBCH 16-18	0.98 ***	-0.40 ns	0.99 ***	0.67 ns	1.00 ***	0.90 ns
BBCH 19	–	–	–	–	0.97 *	0.58 ns
BBCH 30-32	0.97 **	0.76 ns	0.99 ***	0.93 *	0.21 ns	0.84 ns
BBCH 59	–	–	–	–	0.79 ns	0.97 **
BBCH 68-71	-0.36 ns	0.95 **	0.40 ns	0.44 ns	–	–
BBCH 84-89	0.56 ns	0.79 ns	0.74 ns	0.76 ns	0.94 ns	0.72 ns

We also analyzed the extent to which genotypic variations in NUpE or NUtE reflected genotypic variations in NUE_DM using regression analyses (Table 4). During the beginning of the vegetative phase (up to BBCH 31), strong and significant correlations were found between NUE_DM and NUpE under both N conditions ($r > 0.97$ for BBCH 16-18 to BBCH 30-32). From BBCH 59 onwards, the correlation between NUpE and NUE_DM was no more significant, except a weak correlation at harvest for GR18. The opposite pattern was observed for NUtE, with non-significant or weak correlations with NUE_DM up to BBCH 30-32, but higher correlations from BBCH 59 onwards. Thus the genetic variability observed for NUpE during the autumn growth and for NUtE during flowering and seed development can be exploited to tune NUE_DM.

3.4. Deciphering genotypic variation in NUpE-related processes

The above results highlighted NUpE as the main contributor to NUE_DM shaping, especially under N-limiting conditions and as an important driver of its genotype variability during the autumn growth. This raises the question of identifying the key processes underlying this trait. NUpE depends on the quantity of N accumulated in the plant, itself driven by two processes: ability to absorb N per unit of fine-root biomass (SNU) and ability to maximize exchange area with the soil through root system development, that we approximated by fine-root growth.

The QN dynamics of each genotype under low-N conditions (Fig. 4) showed that most N was taken up during the vegetative phase, since genotypes had accumulated 71 to 96% of their final QN by the beginning of flowering, and even mainly during the autumn growth (up to BBCH 32), which already represents 58 to 82% of the total nitrogen absorbed, depending on the genotype (Table S2). However, all genotypes maintained N uptake during the reproductive phase, although the amount and percentage of the N taken up from flowering to seed maturity varied greatly among genotypes (from 4% to 29% for OLESKI and MOHICAN, respectively; Table S2).

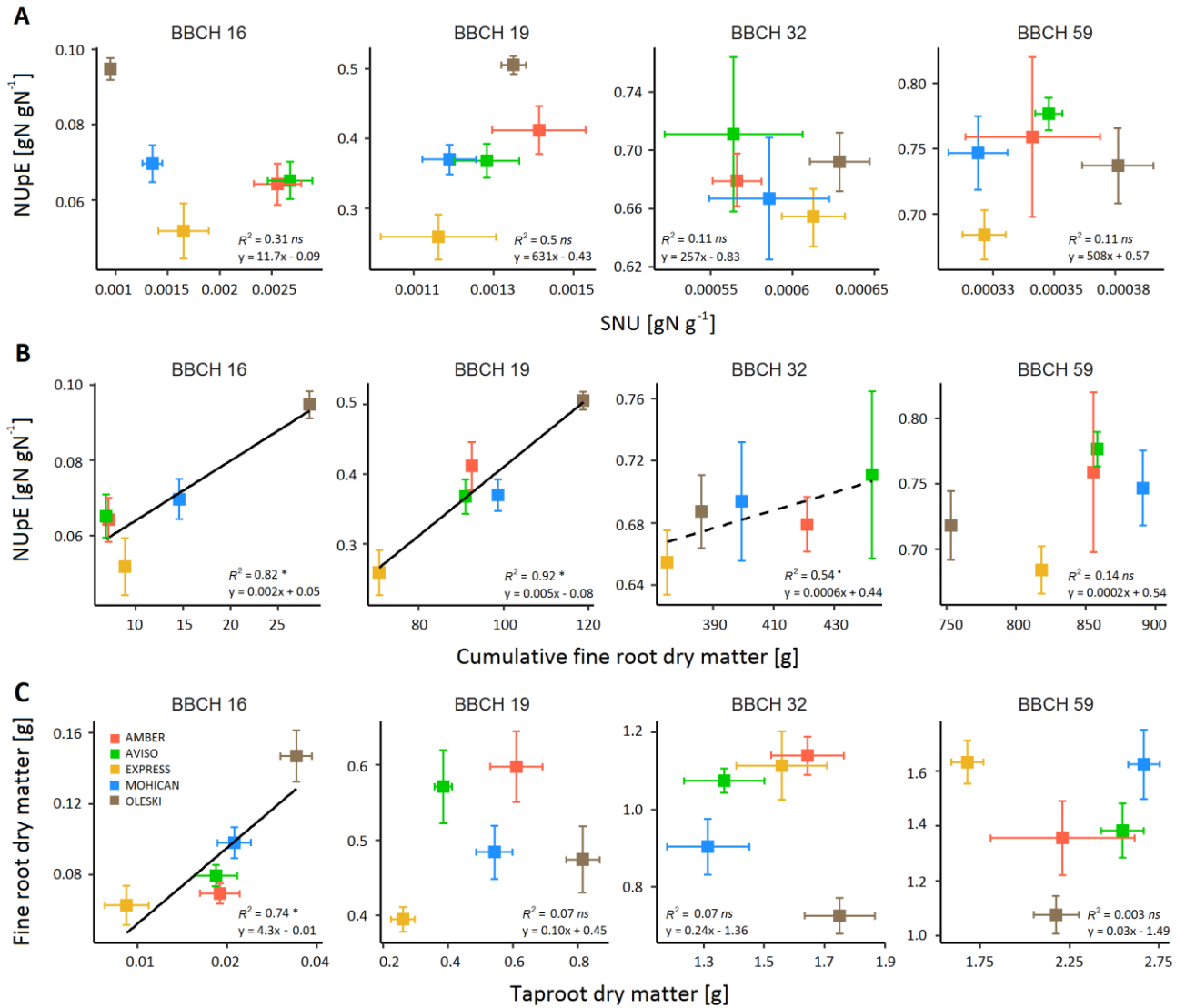


Figure 5. Relationship between (A) NUpE and specific N uptake (SNU), (B) Nitrogen Uptake Efficiency (NUpE) and cumulative fine-root dry matter and (C) fine root and tap root dry matter during the vegetative growth (BBCH 16 to 59) for five winter oilseed rape genotypes under low-N conditions (GR18 experiment). Colored squares indicate the mean value per genotype. Error bars indicate standard errors of the mean. Significant regressions at P -value < 0.05 are indicated by a solid lines and (*) symbol, while significant regressions at P -value < 0.1 are indicated by a dashed line and (.) symbol.

Genotypes did not differ significantly on SNU, except at BBCH 16-18 (Fig. 4). In addition, response curves of SNU obtained for each genotype throughout the crop cycle had no significantly different parameters (Figure S1), and NUpE was never significantly correlated to SNU (Fig. 5A), suggesting that SNU was not the main driver of genotypic nor temporal variability in NUpE. In contrast, significant positive relationships were found between NUpE and cumulative fine-root biomass (Fig. 5B) from the beginning of rosette development (BBCH 16, $R^2= 0.82$) to the beginning of stem elongation (BBCH 32, $R^2= 0.54$). Moreover, fine-root biomass had high genotypic variability during the vegetative phase, but not at seed maturity (Fig. 4, Table S2). Cumulative fine-root biomass could then be considered as a relevant trait for characterizing NUpE variation between genotypes.

As fine roots are tedious to phenotype in the field or on mature plants, we investigated if total- or tap- root biomasses could be used as proxies of fine root biomass. Like fine roots, total- and tap- root biomasses had high genotypic variability during the vegetative phase (Fig. 4, Table S2). No significant correlation was found between fine-root and total root biomasses, nor between fine-root and tap-root biomasses, except at the beginning of rosette development (BBCH 16), when tuberization was low (Fig. 5C). But they exhibited independent genotypic variations, suggesting that genotypes differed in the partitioning of root biomass. Thus, neither taproot nor total root biomass could be used as a proxy of fine-root biomass.

4. DISCUSSION

Our objective was to analyze dynamics of NUE and its components and decipher the processes underlying their genotypic variability, which would merit consideration in breeding programs for low N-input systems. Our study was based on NUE_DM, which we propose as a new variable to monitor NUE shaping accurately throughout the crop cycle. Comparing to the alternative variables already developed (Craswell and Godwin, 1984; Raun and Johnson, 1999; Svecnjak and Rengel, 2006 a), NUE_DM offers the advantage of assessing NUE at each phenological stage and of considering all plant compartments, including fallen leaves, tap roots, and fine roots. NUE studies usually neglect these organs because they are difficult or tedious to harvest, especially in the field or at the plant level. However, our results indicated that the contribution of fine roots and fallen leaves to whole-plant biomass, and thus to NUE, is far from negligible. Under the low-N condition, fine roots represented up to 32% of total biomass during the vegetative phase, and fallen leaves up to 26% at harvest. Regarding N, fine roots and fallen leaves represented up to 21% and 26.5%, respectively, of the N taken up from sowing to harvest. These data are consistent with the results of Malagoli *et al.* (2005 a), who reported a loss of 12% of plant N in fallen leaves. Some other studies assessing NUE include tap roots and sometimes fine roots, but not fully for each individual plant (Hohmann *et al.*, 2016; Yuan *et al.*, 2016, Desbrøll *et al.*, 2016; Ulas *et al.*, 2012) or at each phenological stage, mainly targeting either the seedling or reproductive stages (Louviaux *et al.* 2020; Wang *et al.* 2017; Thomas *et al.*, 2016). To our knowledge, our study is the first to describe the NUE dynamics of winter oilseed rape so completely and accurately, by considering entire individual plants grown under canopy conditions at key phenological stages from sowing to seed maturity.

As the first main finding, our study highlights the early determinism of NUE. Indeed, NUE_DM was a proxy trait of NUE_Seed valid as early as the end of inflorescence emergence (BBCH 59) to discriminate genotypes, and the beginning of stem elongation (BBCH 32) to discriminate N conditions. Correlations were validated for the range of climatic conditions observed in our three experiments, highlighting the robustness of the proxy, even if it should be assessed in more extreme environmental conditions. Thus, for the purposes of genotypic scoring, it may be sufficient to phenotype NUE just before flowering. Some correlations have also been reported in the literature between NUE_Seed and total biomass (Stahl *et al.*, 2019), but only at flowering and not before. Thus, our results highlight the role of the vegetative phase in the determinism of NUE.

Our study is the first to dynamically quantify the relative contribution of NUpE to NUE throughout the crop cycle. Our second main finding is the identification of NUpE as the main contributor to NUE during the whole vegetative phase and particularly during autumn growth. Indeed, up to the beginning stem elongation (BBCH 30-32), NUpE contributed more than 70% to NUE_DM variations, and NUpE genotypic variations strongly correlated to those of NUE_DM. NUE_DM would thus rely mainly on N uptake processes during this period. Accordingly, Cramer (1993) showed that 35% of the total amount of N taken up by the time of harvest had already been taken up by the end of autumn growth. In our study, these values raised to 60 to 80% of the total amount of N, depending on N conditions. This discrepancy could be due to differences in the N balance sheet, which included all plant compartments in our study; and differences in N supply dynamics, which led to higher N availability during the autumn growth, compared to field conditions. Thus, autumn growth was the period during which NUpE strongly determined variations in NUE_DM and also the period during which most of the nitrogen was absorbed.

Interestingly, we also showed that NUpE continued to contribute strongly to NUE after flowering in our experiments, with a relative contribution of 59% under the high-N condition and 73% under the low-N conditions at harvest. Using the same contribution analysis, Kessel *et al.* (2012) on rapeseed and Rakotoson *et al.* (2017) on rice obtained similar ranges of values at harvest, also with a higher relative contribution of NUpE vs NUtE especially under low N conditions. Accordingly, we showed that N uptake continued after flowering, contrary to observations of Malagoli *et al.* (2005 b) but consistent with those of Berry *et al.* (2010), Schulte auf'm Erley *et al.* (2011), and Ulas *et al.* (2012). The proportion of N taken up during the reproductive phase varied according to genotype, but reached up to 29%, which highlights contrasting genotypic behavior in the management of N uptake dynamics, consistent with contrasted NUpE and NUtE values. Thus, NUpE continued to play a significant role during seed development and ripening, especially when N supply was limited.

Even if the contribution of NUtE remained overall low under the low-N condition, NUtE was predominant to explain NUE_DM variations from flowering under the high-N condition. The contributions of NUtE to NUE_DM variations ranged from 41 to 89% and significant correlations between NUE_DM and NUtE genotypic variations were observed as soon as BBCH 59. Kessel *et al.* (2012) also pointed out that the genotype variations in NUE at seed maturity were mainly due to differences in NUtE under high N conditions. These results suggested that N utilization processes balanced N uptake processes during the reproductive phase under plethoric N conditions, *i.e.* when N is largely stored into the plant, which is consistent with previous studies (Girondé *et al.*, 2015 a, b) that have highlighted the importance of genotypic variability in the remobilization processes to improve NUE.

The high N uptake capacities and the poor N remobilization capacities from senescing leaves of rapeseed during vegetative phase during vegetative phase (Dejoux *et al.*, 2000; Malagoli *et al.*, 2005 a; Girondé *et al.* 2015 b) led to the widely held assumption that NUpE is not a limiting factor for increasing NUE (Svecnjak and Rengel, 2006 a; Avice and Etienne, 2014). Our results contradict this assumption, suggesting that N uptake could become a relevant lever for increasing NUE in low N-input systems. In the field, inconsistent results have been reported about drivers of NUE. Some studies report good correlations between NUE and NUpE at harvest (Nyiakako *et al.*, 2014; Stahl *et al.*, 2017), while others, focused on the reproductive phase, explain differences in NUE instead by variations in NUtE (Svecnjak and Rengel, 2006 b; Stahl *et al.*, 2015, 2019). Nonetheless, under low-N conditions, the correlation of NUpE to NUE has always been higher than that of NUtE, (Berry *et al.*, 2010; He *et al.*, 2017), which points out the value of identifying the underlying traits of NUpE, as levers to optimize NUE under low N conditions.

As the last main finding, we showed that the dynamic of fine-root growth was the main driver of genotypic differences in NUpE during the autumn growth, rather than Specific Nitrogen Uptake (SNU). Indeed, genotypes did not differ in SNU after BBCH 16-18 but significantly differed in fine-root growth for all the vegetative phase. In addition, significant correlations were found between NUpE and fine-roots biomass up to BBCH 32, but not between NUpE and SNU. The same results were also observed in the LR15 experiment (data not shown). This absence of genetic variability in SNU constitutes a specific characteristic of winter oilseed rape, since genotypic differences in SNU were reported for *A. thaliana* and *Medicago truncatula* (Richard-Molard *et al.*, 2009; Moreau *et al.*, 2012). In our case, genotypes ran through the same SNU dynamics, even if at different speeds. Thus, in winter oilseed rape, increasing NUE would rely on fine-root plasticity and/or the duration of N uptake, rather than on SNU *per se*, which highlights fine-root biomass as a promising trait for breeding N-efficient cultivars.

Many studies highlighted the crucial role of the root system, and especially fine roots, in N uptake and NUE (Hohmann *et al.*, 2016). However, since recovering all fine roots of winter oilseed rape is impracticable in the field, their study has usually been limited to hydroponic conditions and early developmental stages (Wang *et al.*, 2017; Qin *et al.*, 2019). Under field or field-like conditions, root measurements have usually been limited at best to tap roots (Sieling *et al.*, 2017). Nonetheless, we showed that the ratio of fine- to tap-root biomass differed significantly among genotypes, except at very early developmental stages, when tap roots had barely developed, thus suggesting that taproot DM cannot serve as a proxy of fine-root DM. For breeding purposes, this result clearly highlights the importance of characterizing the fine-root compartment to screen genetic resources. In our study, we considered fine-root biomass as a proxy of fine-root area, but a more detailed genotypic description of root-system architecture (*e.g.* length and number of lateral roots, root diameters, and branching density) could also be relevant. These traits are usually measured at early developmental stages under controlled conditions. We showed that NUE estimated at the end of inflorescence emergence was well correlated with NUE at seed maturity. Thus, phenotyping devices that can phenotype root-system architecture accurately up to the BBCH 59 stage would be valuable for screening genetic diversity. Consequently, the next challenge for phenomics would be to extend the duration of growth supported by the existing high-throughput phenotyping platforms (Jeudy *et al.*, 2016) up to this developmental stage.

SUPPLEMENTARY DATA

Table S1. Winter oilseed rape genotypes investigated, by experiment, release year and type of variety. Experiment codes: LR15, Le Rheu 2014-2015; GR15, Grignon 2014-2015; GR18, Grignon 2017-2018. Type codes: ++', high glucosinolate and erucic acid contents; 00', low glucosinolate and erucic acid contents.

Genotype	Experiment	Year of release	Type
AMBER	LR15, GR18	2003	00'
ASTRID	LR15	2004	00'
AVISO	LR15, GR15, GR18	2000	00'
EXPRESS	LR15, GR18	1993	00'
MOHICAN	LR15, GR18	1995	00'
MONTEGO	LR15	2001	00'
OLESKI	GR18	<1980	++'

Table S2. Total plant N quantity, N Uptake- and Utilization- Efficiencies, and tap- and fine-root biomasses of five winter oilseed rape genotypes grown under the low-N condition (GR18 experiment) at five sampling dates. Means with the same letter for a given sampling date do not differ significantly according to Tukey's test (P -value ≤ 0.05).

Trait / Stage	Unit	Genotype				
		AMBER	AVISO	EXPRESS	MOHICAN	OLESKI
Whole plant N-quantity	gN plant ⁻¹					
BBCH 16		0.02 a	0.02 a	0.01 a	0.02 a	0.03 b
BBCH 19		0.13 bc	0.12 b	0.08 a	0.12 b	0.16 c
BBCH 32		0.24 a	0.25 a	0.20 a	0.23 a	0.24 a
BBCH 59		0.29 a	0.30 a	0.26 a	0.29 a	0.28 a
BBCH 89		0.32 ab	0.36 ab	0.29 ab	0.40 a	0.30 b
N Uptake Efficiency (NUpE)	gN gN ⁻¹					
BBCH 16		0.06 a	0.07 a	0.05 a	0.07 a	0.09 b
BBCH 19		0.41 ab	0.37 b	0.26 c	0.37 b	0.51 a
BBCH 32		0.68 a	0.71 a	0.66 a	0.67 a	0.69 a
BBCH 59		0.76 a	0.78 a	0.68 a	0.75 a	0.74 a
BBCH 89		0.65 ab	0.74 ab	0.61 ab	0.83 b	0.61 a
N Utilization Efficiency (NUE)	g gN ⁻¹					
BBCH 16		19.8 a	19.8 a	19.7 a	20.0 a	20.6 a
BBCH 19		31.6 a	29.4 ab	26.8 b	28.8 ab	28.1 ab
BBCH 32		37.9 a	34.1 bc	35.5 b	34.2 bc	32.6 c
BBCH 59		47.3 a	54.1 b	43.9 a	55.2 b	42.1 a
BBCH 89		92.8 a	86.7 a	80.3 ab	86.9 a	76.2 b
Total root dry matter	g plant ⁻¹					
BBCH 16		0.09 ab	0.10 ab	0.08 b	0.12 a	0.18 c
BBCH 19		1.16 a	0.96 ab	0.62 b	1.03 a	1.29 a
BBCH 32		2.78 a	2.44 a	2.67 a	2.22 a	2.48 a
BBCH 59		3.57 ab	3.93 ab	3.31 a	4.29 b	3.25 a
BBCH 89		3.18 a	3.29 a	1.68 b	2.87 a	1.93 b
Taproot dry matter	g plant ⁻¹					
BBCH 16		0.02 ab	0.02 b	0.01 c	0.03 ab	0.03 a
BBCH 19		0.61 ab	0.39 cd	0.26 d	0.54 bc	0.82 a
BBCH 32		1.64 a	1.37 a	1.56 a	1.31 a	1.75 a
BBCH 59		2.21 ab	2.54 a	1.68 b	2.67 a	2.18 ab
BBCH 89		2.20 a	2.48 a	1.07 b	2.26 a	1.25 b
Fine root dry matter	g plant ⁻¹					
BBCH 16		0.07 a	0.08 a	0.06 a	0.10 a	0.15 b
BBCH 19		0.55 ab	0.57 a	0.36 b	0.48 ab	0.47 ab
BBCH 32		1.14 a	1.07 a	1.11 a	0.90 ab	0.73 b
BBCH 59		1.36 ab	1.38 ab	1.63 a	1.62 a	1.08 b
BBCH 89		0.97 a	0.81 a	0.61 a	0.62 a	0.68 a

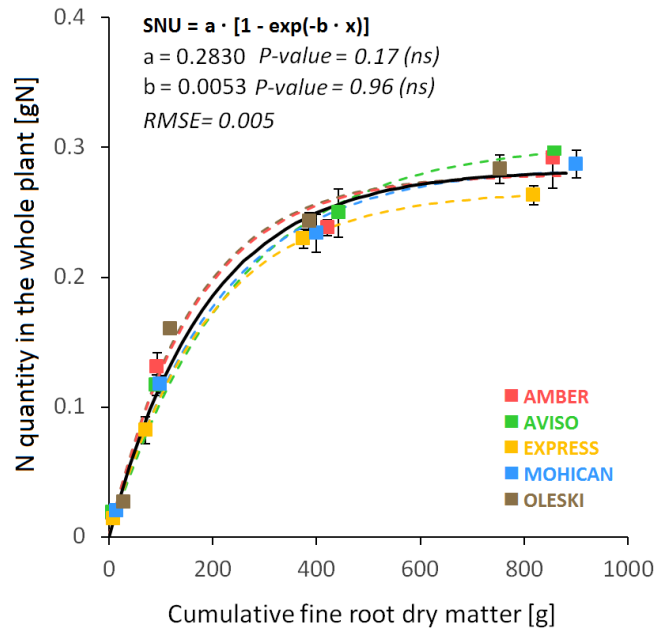


Figure S1. Dynamics of Specific N Uptake (SNU) for five winter oilseed rape genotypes grown under the low-N condition during the vegetative growth (GR18 experiment). The general response curve of SNU expressed as the amount of N in the plant per g of cumulative fine-root biomass. was fitted by a negative exponential function. Dashed lines represent individual genotypes. while the solid black line represents the mean of all genotypes. Colored squares indicate the mean value per genotype for the measured amount of N in the whole plant and the estimated amount of cumulative fine-root dry matter. The P -values correspond to the comparison of model parameters between genotypes and RMSE to the quality of the model. Error bars depict standard errors of the mean.

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Overview and highlights of Chapter III

In this chapter, we have studied NUE-related traits during vegetative growth that report on the genotypic variability of NUE and ultimately on seed yield at harvest. To this end, we selected contrasting winter oilseed rape genotypes for seed yield response to nitrogen inputs, and we analyzed the kinetics of growth, biomass, and nitrogen content of shoots and roots over the whole plant cycle. As NUE at seed harvest is the result of plant growth, N-availability, and yield elaboration over the crop cycle, we calculated NUE at the main stages of the growing cycle, with consideration of the whole plant biomass produced since sowing (NUE_DM).

Because NUE defined as seed yield per unit of soil N available at harvest indicated a strong correlation with NUE_DM at harvest, we investigated the quality of this correlation at different previous stages along the crop cycle. We looked at the earliest stage in which NUE_DM correlated with the harvest point. We evidenced that NUE_DM measured at the beginning of stem elongation (BBCH 32) could be used as an early and robust proxy trait to characterize N-response at harvest. However, to discriminate genotypes, NUE_DM should be measured as early as the end of the vegetative growth (BBCH 59). We evidenced that the variation in NUpE explained the variation in NUE_DM better than NUtE during autumn growth for both low and high N-conditions. However, we observed a switch in NUpE and NUtE contributions to NUE_DM from flowering but with different trends dependent on N-conditions. Whereas at high-N NUtE mainly contributed to NUE_DM variation during the reproductive phase, NupE mainly contributes at low-N. Nevertheless, under high-N NupE became still relevant and should not be neglected.

We studied the genotypic variation of NUE_DM and its components, and we highlighted a higher genotypic effect during the vegetative at low N-supply. Accordingly, NupE was the main driver of NUE_DM genotypic variability during autumn growth, which led us to assess the key processes underlying this trait and their genotypic variability. The observed genotypic variability in NUpE was not due to specific N uptake but to fine root growth. The accumulation of the fine root biomass over the growth cycle was positively correlated with NUpE, which highlighted cumulated fine root biomass as an early trait determining nitrogen use efficiency.

Our results suggested a gap between the ability of NUE_DM to predict NUE genotypic variations at seed maturity (valid from BBCH 59) on the one hand, and on the other hand, the role of NUpE and fine-root growth as the main determinants of NUE_DM (valid during autumn growth). Thus, to fully optimize genotypic performance in NUE, it would be necessary to better understand the changes that occur in the plant during stem elongation (gap period). A modeling approach might be useful to understand and rank the importance of the processes related to C-N acquisition and partitioning, which are modified greatly by the development of new organs during this period.

CHAPTER IV.

Which efficiencies explain oilseed rape genotypic variations in biomass accumulation and partitioning under low N-availability?

A model assisted phenotyping approach.

In Chapter III, we highlighted NUE_DM as a proxy trait of NUE_Seed, allowing to discriminate genotypes from BBCH 59 onwards and N effects from BBCH 32 onwards. NUE_DM directly rely on whole plant biomass elaboration and Chapter II highlighted that processes leading to total biomass elaboration in response to N-conditions are regulated by the C and N fluxes in the whole plant. Therefore, we suggested that N-use efficiency might be assessed from vegetative phase using a conceptual framework of whole plant C-N functioning that report the dynamics of biomass elaboration during the growth cycle.

In Chapter II, we pointed out that root traits might drive NUpE under low-N conditions. Chapter III identified the dynamic of fine-root growth as the main driver of genotypic differences in NUpE during the autumn growth. However, phenotyping the genotypic variation in NUpE and fine root growth remains tricky, especially under field conditions. A model-assisted phenotyping approach might better understand biomass partitioning into the shoot and the root compartments in response to nitrogen and to genotype and therefore simulate fine root biomass instead of measuring it.

This chapter proposed a whole-plant compartmental conceptual model describing whole plant C and N interactions in winter oilseed rape. We proceed using three steps. First, we proposed a whole-plant conceptual modeling framework for C-N functioning of winter oilseed rape. Second, we assessed the validity of the relationships that were adjusted for rosette growth, during stem elongation and then during the reproductive phase. Third, we used the developed framework to investigate parameter variation in response to N-conditions and to assess the genotypic variation under low-N conditions. Can such a framework be used to reduce the range of genotypic variation in the processes underlying biomass production in response to N nutrition levels?

This chapter has been written as a scientific paper, for the purpose of a forthcoming submission.

1. INTRODUCTION

In an agro-ecological context of reducing nitrogen (N) inputs, breeding winter oilseed rape cultivars that are efficient in uptake and use of N to ensure high seed yields under lower N-inputs constitutes a core issue in ensuring the sustainability and competitiveness of this crop (Bouchet et al. 2016; Louvieaux et al. 2020). One key lever relates to the improvement of Nitrogen Use Efficiency (NUE), which represents the plant capacity to valorize N-inputs in terms of biomass or yield (Moll et al., 1982).

However, improving N-use efficiency through breeding remains a challenge, due to the complexity of oilseed rape functioning and its plasticity in response to N-conditions. NUE relies on biomass accumulation in seeds, itself depending on biomass accumulation and partitioning at the whole plant scale. Oilseed rape biomass accumulation is a dynamic process which results from plant growth and development, soil N-availability, and whole-plant N-fluxes. It involves a set of closely interconnected traits dealing with the regulation of C and N uptake and allocation in the whole-plant. Plant N-uptake is related to root system growth and spatial arrangement (root system architecture), and to root physiological functions such as nutrient absorption, transport and metabolism (Lynch, 1995; Pagès et al., 2014; Lynch et al., 2015; Shi et al., 2018). The carbon input is related to photosynthesis as well as to light interception, which depends on shoot architecture, (*i.e.* spatial arrangement of the phytomers) and dynamic of leaf area expansion (Jullien et al., 2011). Differential C utilization in shoot and roots depends on plant N-status (Justes et al. 2000; Colnenne et al. 2012). Furthermore, plant response to N-availability displays wide genetic and environmental variability in several crops such as maize (Reymond et al., 2003), wheat (Gaju et al., 2011), oilseed rape (Girondé et al., 2015) and rice (Rakotoson et al., 2017). Another complexity derives from the dynamic variation of phenotypes over the growing cycle (Granier et al., 2014), which requires thorough knowledge of traits related to development and growth as well as a characterization of their genetic variability (Ulas et al., 2013). Thus, identifying and hierarchizing pertinent traits to decipher oilseed rape response to N-availability requires a whole-plant approach.

One possible approach to understand plant response to N-availability is to break down complex traits, such as NUE, as the sum of variation in simpler constituent variables (Thurling, 1991). However, as the constituent variables are often interconnected, an ecophysiological model could be useful to formalize the relationships between them and study the plant system as a whole, in contrast to study constituent variables independently. A modeling framework would thus constitute a useful approach to integrate impacts of individual variables and lead to the whole-plant or canopy integrative phenotype (Moreau et al. 2012a; Ghanem et al., 2014; Granier and Vile, 2014; Chenu et al., 2017, Tardieu et al., 2017). Using model parameters to screen genotypic or N variability presents also a major advantage. Indeed, by construction, parameters ought to be independent of the environmental noise and may exhibit narrower variation than associated state variables (Martre et al., 2015). Thus, the use of model parameters as traits to study genotypic and N effects will help prioritize the processes underlying complex variable elaboration in a given N- environment and highlight those valuable for breeding. Therefore, plant and crop models are powerful tools for assessing and predicting the environmental impact on plant functioning and identifying new breeding traits (Muller and Martre, 2019).

Such model-assisted phenotyping approaches have been successfully used to deal with genotype x environment (G x E) interactions and to hierarchize the main physiological processes responsible for phenotypic differences according to abiotic constraints for different species such as barley (Yin et al., 1999), maize (Reymond et al., 2003; Chenu et al., 2009), wheat (Laperche et al., 2006; Semenov et al., 2007; Bertin et al., 2010), *Arabidopsis thaliana* (Richard-Molard et al., 2009) and *Medicago truncatula* (Moreau et al., 2012b).

Functional and structural plant models (FSPM) provide an explicit representation of plant architecture in addition to plant functioning. Therefore, they are particularly suitable for studying the heterogeneity of resource capture and assimilate utilization in space and time (Dejong et al., 2011) and predicting plant growth under variable environmental conditions (Tian et al., 2017). Most existing FSPMs focus either on the above- or below-ground plant compartments and only a few consider the whole crop cycle, as recently reviewed by Muller and Martre (2019). Nevertheless, Louarn and Faverjon (2018) developed a individual-based model to describe shoot and root morphogenesis, C and N acquisition, and population dynamics in forage legumes. In oilseed rape, Jullien et al (2007, 2011) developed an architectural model simulating the dynamics of organ growth from sowing to harvest, but it was limited to above-ground. Böttcher et al. (2020) published a complex oilseed crop model based on more than forty parameters to simulate dry matter production and partitioning and N uptake and N distribution under optimal and limiting conditions but that are difficult to measure at the plant level. To our knowledge, no FSPM model describing N and C fluxes between the shoot and root compartments to simulate whole-plant biomass accumulation during the whole crop cycle has been published yet for winter oilseed rape.

In the *Brassicaceae* family, Richard-Molard et al. (2009) developed a simple whole-plant functional model on *Arabidopsis thaliana* based on twelve parameters, called ARNICA, and used it to characterize the effects of N nutrition and genotype variation on whole-plant C and N functioning and shoot and root biomass accumulation. This model is based on interactions between the N and C fluxes and offers a dynamic description of root growth and leaf area expansion during the rosette growth. However, it was dedicated to plants grown under controlled conditions with a constant regime of air temperature and light. Such a modeling approach could be valuable for oilseed rape to understanding the dynamics of C and N resource acquisition and assimilates allocation. Although oilseed rape is phylogenetically close to *Arabidopsis thaliana*, it has a more complex growth cycle and a tuberized root system, which acts as a reservoir for nutrients and assimilates and differs from fine roots, which support water and nutrient uptake and have the ability to branch out and proliferate in soil layers (Malagoli et al. 2005a).

The objective of this study was to set an ecophysiological modeling framework dedicated to winter oilseed rape growth and C-N functioning, and to use it to determine the main parameters describing oilseed rape responses to N-availability and supporting the observed genotypic variation in biomass accumulation under low N-conditions. To achieve this, we orchestrated a three-step approach. First, we proposed a whole-plant conceptual modeling framework for winter oilseed rape. Second, we assessed the model validity during rosette growth period, during stem elongation up to flowering and during the reproductive phase. Third, we investigated the variation of the model parameters in response to N availability and to genotype variation under low-N conditions, by using the validated conceptual model.

Table IV.1. Characterization of the environmental conditions for the developmental periods of both GR15 and GR18 experiments (GR15, Grignon 2014-2015; GR18, Grignon 2017-2018). All stages and periods were estimated using the average values of the AVISO development. The cumulative N available in pots for plant growth includes mineral N initially present in the substrate and N supplied by fertilization.

Developmental period	Autumnal growth	Winter period	Spring growth (stem elongation)	Flowering period	Seed filling	Total cycle
BBCH stages	sowing - BBCH19	BBCH19 - 32	BBCH32-60	BBCH60-69	BBCH69-89	sowing-BBCH89
Duration (days after sowing)						
GR15	83	99	31	11	66	294
GR18	88	76	47	18	57	297
Accumated growing degree days (°C)						
GR15	1039	462	242	142	950	2841
GR18	899	390	358	241	950	2828
Cumulative PAR (J cm⁻²)						
GR15	32495	22883	17545	12373	68907	15280
GR18	30129	14817	22122	16613	62388	14450
Cumulative rainfall (mm)						
GR15	127	158	34	0	81	399
GR18	149	183	110	23	142	607
Cumulated N-avaible (gN plant⁻¹)						
GR15 - N0	0.24	0.06	0.02	0.02	0.11	0.45
GR15 - N1	0.29	0.11	0.04	0.04	0.22	0.70
GR15 - N2	0.52	0.34	0.11	0.11	0.67	1.75
GR18 - N0	0.27	0.03	0.03	0.03	0.07	0.44
GR18 -N2	0.71	0.26	0.26	0.26	0.51	1.99

2. MATERIAL AND METHODS

2.1. Site description and plant material

Two experiments were carried out at Thiverval-Grignon (48°50'21.7"N; 1°56'48.4"E), France, during the 2014–2015 and 2017–2018 cropping seasons (hereafter referred to as GR15 and GR18, respectively). Plants were grown under up to three contrasting N-conditions as described in detail in Table IV.1. Data on average air temperature (°C), rainfall (mm), and photosynthetically active radiation (PAR; J cm⁻²) were recovered daily throughout the whole crop cycle using the INRAE CLIMATIK platform (<https://intranet.inrae.fr/climatik>). The cumulated growing degree-days were calculated from sowing, assuming a base temperature of 0 °C, as supported by Colnenne et al. (2002) and Dresbøll et al. (2016). These data allowed identifying five growing periods: autumnal growth, winter period, spring growth, flowering period, and seed filling. Phenological stages were identified using the winter oilseed rape BBCH scale (Lancashire et al., 1991). Duration, cumulated growing degree days, accumulated PAR, cumulated precipitations, and N-availability (N_{soil} + N_{supply}) for each growing period are summarized in Table IV.1.

Plant material was composed of five winter oilseed rape (*Brassica napus* L.) genotypes. Genotypes were chosen for their contrasting seed yield response to N-nutrition conditions, dynamics of shoot biomass accumulation, and amount of N absorbed during the growing cycle, according to previous experiments conducted in a French field network (Bouchet et al., 2016). In the GR15 experiment, one genotype (AVISO) was investigated under three contrasting N-conditions, whereas in the GR18 experiment five genotypes (AMBER, AVISO, EXPRESS, MOHICAN, and OLESKI) were investigated under low-N condition and one genotype (AVISO) under low- and high-N conditions.

2.2. Experimental design

Experiments were designed to test either N or genotype factors using a randomized complete block design, with six and seven replicates for GR15 and GR18 experiments, respectively. Plants were sown on individual tubes of 1 m in height and 0.16 m in diameter, grouped into containers of 1 m³, and placed outdoors; they were therefore submitted to field-like climate (PERISCOPE device, Bissuel-Belaygue et al., 2015). Each tube was filled with a mixture of attapulgite and clay pebbles (50:50, v/v), achieving a bulk density of 0.52 g cm⁻³, and were regularly supplied with a nitrogen-free Hoagland solution (Hoagland and Arnon, 1939). Nutrient solution inputs were adjusted to maintain substrate moisture at 80% of the field water capacity. In the containers, the space between columns was filled with soil to ensure thermal insulation of the root system. Two rows of border plants were planted around the tubes to mimic field bioclimatic conditions. Six seeds of similar weight were sown in each tube. After seedling thinning out, one single plant per column was retained, resulting in a homogeneous canopy of 35 plants m⁻², grown up to harvest. Pests and diseases were controlled through applications of insecticides and fungicides.

Table IV.2. Overview of the destructive samplings carried out in the 2014-2015 and 2017-2018 Grignon experiments (GR15 and GR18, respectively) and qualitative estimation of the main plant organs present at each growing phase. In the GR15 experiment, a total of six intermediate samplings were carried out: four during rosette growth and the beginning of stem elongation (until BBCH 30-32), one at the end of stem elongation, one just before flowering (BBCH 59), and one at the beginning of pod formation (BBCH 71). In the GR18 experiment, three intermediate samplings were carried out during rosette growth and one at the end of the vegetative growth. An additional sampling at seed maturity (harvest) was carried out in both experiments.

Growing phase BBCH stage	Leaves development and rosette growth					Stem elongation 59	Reproductive phase	
	16	19	20	21	30-32		71	88-89
Samplings								
GR15		x	x	x	x	x	x	x
GR18	x	x			x	x		x
Plant organs								
Taproots	■					■	■	
Fine Roots	■					■	■	■
Green leaves	■					■	■	■
Fallen leaves	■	■				■	■	
Main Stem	■					■	■	
Branches	■					■	■	
Pods	■					■	■	

Color scale

The color scale refers to the presence of the organ under consideration at the dedicated growing phase and among genotypes. Black (++) all plants presented the considered organ, dark grey: most of the plants presented the considered organ, light grey: some of the plants presented the considered organ, white: the organs were absent for all plants.

2.3. Nitrogen management

Nitrogen nutrition was provided through a solution of KNO_3 and $\text{Ca}(\text{NO}_3)_2$ (1:1 valence) mixed with the N-free Hoagland solution (Hoagland and Arnon, 1939), to ensure uniform nitrogen availability within each tube. The N-solution was supplied every 200 growing degrees day from emergence to harvest, with a total of 14 N-supplies over the growth cycle. The amounts of N applied per tube were calculated to generate up to three contrasting crop N status conditions. At the end of the experiment, the low-N condition (N0) corresponded to a total of $0.45 \text{ gN plant}^{-1}$ for GR15 and $0.44 \text{ gN plant}^{-1}$ for GR18 (equivalent to 50 and 49 kg N ha^{-1} in the field, respectively). The moderate-low N-condition (N1) was only tested in the GR15 experiment and corresponded to $0.7 \text{ gN.plant}^{-1}$ (equivalent to 78 kgN ha^{-1}). The high-N condition (N2) corresponded to $1.75 \text{ gN plant}^{-1}$ for GR15 and to $1.99 \text{ gN plant}^{-1}$ for GR18 (equivalent to 196 and 223 kgN ha^{-1} , respectively).

Substrate moisture and its NO_3^- and NH_4^+ concentrations were measured at three different substrate layers (0–30, 30–60, and 60–90 cm), and at each sampling date (refer to the above paragraph) for both experiments and corresponded to six periods (autumnal growth, winter period, beginning of the stem elongation, flowering initiation, seed filling and seed maturity). Mineral N initially present in the substrate (N_{soil}) was measured using the Kjeldahl method (Kjeldahl, 1883). Using this data, we calculated the amount of mineral N available during the growing cycle ($N_{\text{soil}} + N_{\text{supply}}$).

2.4. Sampling and measurements

Some intermediate samplings were performed, in addition to the final harvest at seed maturity (Table IV.2). In the GR15 experiment, a total of six intermediate samplings were carried out: four during rosette growth and the beginning of stem elongation (until BBCH 30-32), one during stem elongation, just before flowering (BBCH 59), and one after flowering during pod formation (BBCH 71). In the GR18 experiment, three intermediate sampling dates were carried out during rosette growth (BBCH16, BBCH19, BBCH32) and one at the end of vegetative growth (BBCH59). In GR15 and GR18 experiments, 6 and 7 to 8 replicates were sampled respectively.

At each sampling date, harvested plants were separated into tap roots, fine roots, green leaves, senescing and fallen leaves, main stem, lateral branches, pods including immature seeds, seeds and pod walls when pods were dehiscent. In the GR18 experiment, green and fallen leaf areas were determined by a LI-COR 300 (Lincoln, NE, USA) and pod area was determined by image analysis using the ImageJ software (Rasband, 1997). In the GR15 experiment, fallen leaf area was not measured but was estimated as a function of the measured green leaves area (Figure IV.S2). We approximated the main stem area by calculating the area of a cone, determined by a basal diameter corresponding to the diameter measured at the base of the main stem and by a height equal to the total length of the main stem. Lateral branches were not considered in this estimation. The total pod area was estimated by doubling the projected area, as suggested by Jullien et al. (2010), assuming pod as a flat organ whose two faces photosynthesize. Each plant fraction was lyophilized and weighed to obtain the dry weight. Samples were ground to a fine powder and subsequently analyzed for carbon and nitrogen content according to the Dumas combustion method (Dumas, 1826; Buckee, 1994) using a C-N analyzer (Vario MICRO Cube, Elementar France). The C and N contents of each sample were multiplied by their corresponding dry matter to determine the total C and N quantity of each plant fraction respectively.

Table IV.3. Measured and/or calculated traits at the end of the experiment, with the abbreviation used in experiments GR15 and GR18, full name of the trait, measurement description and fractions included for calculation and units.

Trait Abbreviation	Trait full name	Description of the compartments / Formula	Units
Measured state variables			
SDM	Shoot Dry Matter	Dry Biomass of green and fallen leaves, stems, pod wall and seeds	gDM plant ⁻¹
RDM	Root Dry Matter	Dry Biomass of taproots and fine roots	gDM plant ⁻¹
Plant DM	Total Plant Dry Matter	Dry Biomass of the whole plant (SDM+RDM)	gDM plant ⁻¹
QNS	Total Shoot N-quantity	N-quantity of green and fallen leaves, stems, pod wall and seeds	gN plant ⁻¹
QNR	Root N-quantity	N-quantity of taproots and fine roots	gN plant ⁻¹
QNP	Total Plant N-quantity	N-quantity of the whole plant (QNS+QNR)	gN plant ⁻¹
QCS	Total Shoot C-quantity	C-quantity of green and fallen leaves, stems, pod wall and seeds	gC plant ⁻¹
QCR	Root C-quantity	C-quantity of taproots and fine roots	gC plant ⁻¹
QCP	Total Plant C-quantity	C-quantity of the whole plant (QNS+QNR)	gC plant ⁻¹
GLA	Green leaves Area	Green leaves area per plant	cm ² plant ⁻¹
FLA	Fallen Leaves Area	Dead leaves area at falling	cm ² plant ⁻¹
LA	Leaf Area	Sum of green leaves area and fallen leaves area	cm ² plant ⁻¹
-	Plant Pod Area	(Lateral area of each pot · 2) · pod number per plant	cm ² plant ⁻¹
-	Main stem area	Main stem area per plant	cm ² plant ⁻¹
PA	Plant Area	Sum of green and fallen leaves, main stem and pods area	cm ² plant ⁻¹
Calculated state variables			
FineRDM _{cum}	Cumulative fine root Dry Matter	Integral of the fine root biomass	g plant ⁻¹
GPA _{cum}	Cumulative Green Plant Area	Integral of the green surfaces (leaves, main stem and pods)	cm ² plant ⁻¹
Model efficiencies			
QNR/QNP	Part of QNP allocated to roots	Root N-quantity = a(Plant N-quantity)	-
QNS/QNP	Part of QNP allocated to shoots	Shoot N-quantity = a(Plant N-quantity)	-
QCR/QCP	Part of QCP allocated to roots	Root C-quantity = a(Plant C-quantity)	-
QCS/QCP	Part of QCP allocated to shoots	Shoot C-quantity = a(Plant C-quantity)	-
QCStem/QCS	Part of QCS allocated to stems	Stem C-quantity = e ^(a+bx)	-
FLA/PA	Fallen leaves area ratio	Fallen leaves area = a(Plant area)	-
PA/QNS	Conversion of QNS into PA	Plant area = a(Shoot N-quantity)	cm ² gN ⁻¹
SCA	Specific Carbon Assimilation	(QCR + QCS) / Cumulative GPA	[gC cm ²] ⁻¹
SNU	Specific Nitrogen Uptake	(QNR + QNS) / Cumulative fine RDM	gN g ⁻¹
FineR/RDM	Fine root ratio	Fine Root DM = a(Total Root DM)	-
NUE-components			
N-available	Nitrogen available for the plant	Nsoil + Nsupply	gN plant ⁻¹
NUE	Nitrogen Use Efficiency	Total PlantDM / N-available	g gN ⁻¹
NUtE	Nitrogen Utilization Efficiency	Total PlantDM / (QNR+QNS)	g gN ⁻¹
NUpE	Nitrogen Uptake Efficiency	(QNR+QNS) / N-available	gN gN ⁻¹

2.5. State variables calculation, cumulative variable estimation, and global indicators.

State variables were calculated either per plant fraction (tap roots, fine roots, green leaves, senescing and fallen leaves, stems, and pods), per plant (all plant fractions combined), per shoot part (above-ground plant fractions including green leaves, fallen leaves, stems, and pods), or per root part (including tap and fine roots) (Table IV.3).

Total shoot dry matter (**SDM**) corresponded to the sum of the biomass of green leaves, fallen leaves, stems, and pods per plant, whereas the root dry matter (**RDM**) corresponded to the sum of the tap and fine root dry matters per plant. The total plant dry biomass (**Total Plant DM**) was the sum of the belowground and aboveground biomasses of the plant, including all leaves previously fallen. The total shoot N-quantity (**QNS**) corresponded to the sum of the N-content of each compartment multiplied by its dry matter, including fallen leaves, whereas the root N-quantity (**QNR**) corresponded to the sum of the tap roots N-content multiplied by their dry matter and of the fine roots N-content multiplied by their dry matter. The total shoot carbon quantity (**QCS**) and the root carbon quantity (**QCR**) were calculated in the same way. The total plant area (**PA**) was the sum of the generated areas of all leaves (green, senescing and fallen leaves), pods, and main stem (excluding branches) of each individual plant.

Moreover, to link the dynamics of model variables and the temporal samplings, we need to estimate some cumulative variables related to shoot and root compartments. The cumulative fine root biomass (**FineRDM_{cum}**) was computed as the area under the curve of fine root biomass dynamics obtained from the destructive measurements of fine root dry matter (Supplementary Figure IV.S3). Symmetrically, the cumulative green leaf area was computed from the destructive measurements of the green leaf area during the crop cycle. Two Gaussian functions were used to fit the dynamics of green (leaves, main stem and pods) plant area (**GPA_{cum}**) for each N-condition and genotype (Supplementary Figure IV.S4), a first from emergence to winter and a second from winter to harvest (Justes et al., 2000) with the following equation [Eq. 1].

$$f(x) = ae^{1 - \frac{(t-b)^2}{2c^2}} \quad \text{[Equation 1]}$$

Parameters a, b, and c were adjusted to minimize the sum of squares deviation using the Generalized Reduced Gradient method for nonlinear optimization (Lasdon et al., 1973). The integral of the fitted curve, representing the cumulative green plant area, was approximated using Riemann's sum. C and N-efficiencies were calculated as described in Table IV.3.

Last, to evaluate the global behavior of the genotypes under the chosen N condition, we introduced two indicators. As suggested by Lecarpentier et al. (pers. comm.), we considered plasticity to nitrogen availability as the ability of one genotype to exhibit different trait (Y_t) values in two contrasting nitrogen environments (absolute plasticity, Eq. 2).

$$\text{Absolute plasticity} = \left| \frac{Y_T^{N2} - Y_T^{N0}}{Y_T^{N2}} \right| \times 100 \quad \text{[Equation 2]}$$

Similarly, genotypic variation was defined as the range of trait variation (Y_t) between the highest and the lowest value observed among the genotypes studied in a given N-condition (absolute genotypic variation, Eq. 3).

$$\text{Genotypic variation} = \left| \frac{Y_T^{\max} - Y_T^{\min}}{Y_T^{\max}} \right| \times 100 \quad [\text{Equation 3}]$$

2.6. Statistical analysis

All statistical analyses were carried out using the R software (RCoreTeam X R version 3.4.2, <http://www.R-project.org/>, 2013).

Linear regression models were fitted with the `lm()` function. Linear relationships were evaluated by estimating the slope (a), intercept (b) and coefficient of determination (R^2) of the regression ($y = a + bx$). When the intercept was not significantly different from zero (p -value > 0.05) we adjusted the following linear model $y = ax$ with the intercept (b) fixed to zero. Parameters of nonlinear models (*ie.* logistic curves and exponentials) were adjusted using the `nls()` function (Bates et al. 1998, 1992).

The accuracy of the adjusted model was analyzed by computing the Root Mean Square Error (RMSE, Eq 4), where X_i is the value of the i -th observation and y_i is the value of the i -th prediction and n is the number of observations.

$$RMSE = \sqrt{\frac{\sum (X_i - y_i)^2}{n}} \quad [\text{Equation 4}]$$

Type II analysis of variance (ANOVA) was carried out on the state variables using the R statistical package 'car'. ANOVA assumptions were tested using the Shapiro-Wilk and Levene's test. Significance of slopes between genotypes and/or nitrogen efficiency parameters was tested using analyses of covariance (ANCOVA). The Hotelling's T-Squared distribution test was used to test the parameters of the nonlinear models (Hotelling, 1931). Statistical significance was estimated at $\alpha = 5\%$. The 95% confidence intervals were computed using the `predict()` function (Chambers et al. 1992).

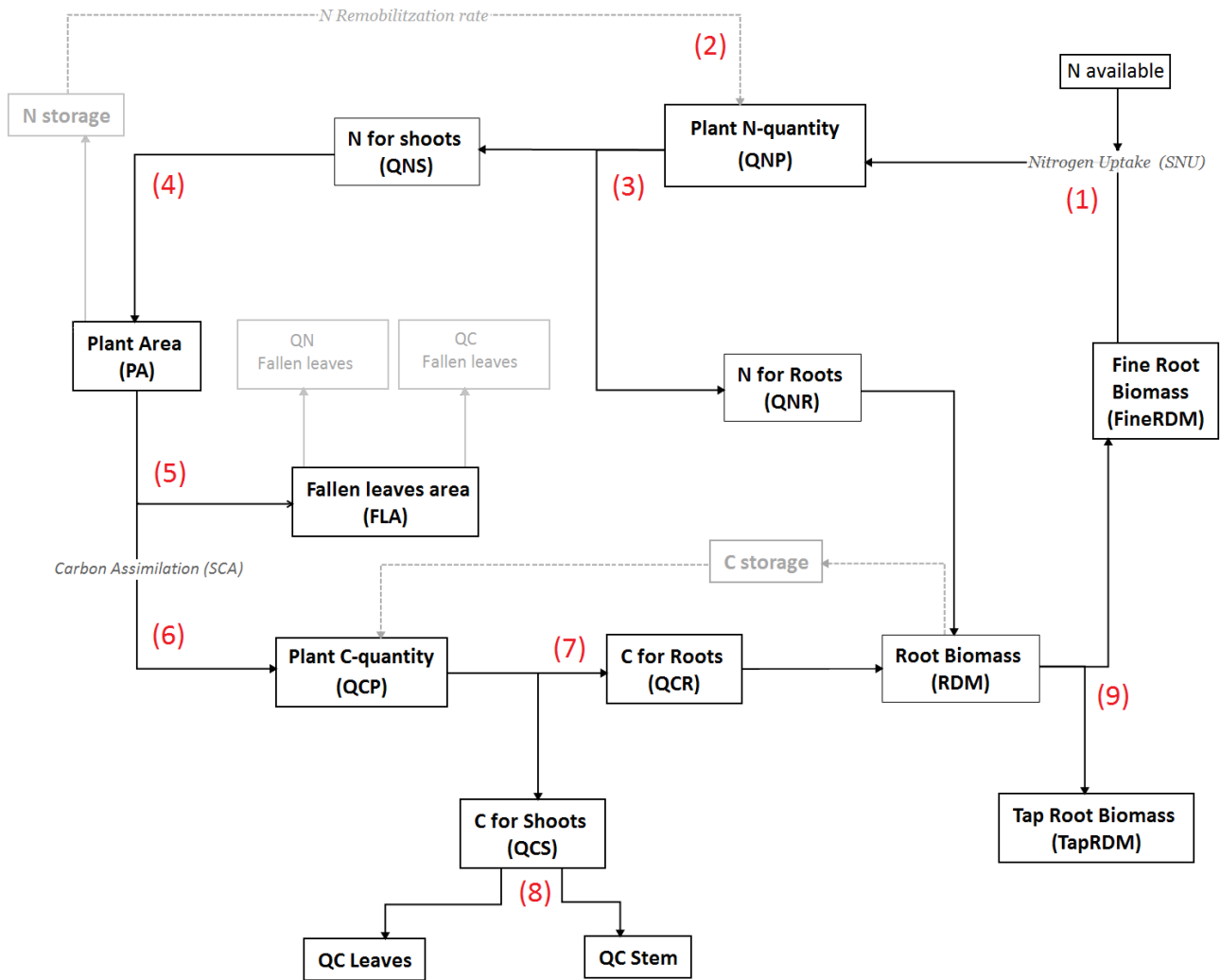


Figure IV.1. Winter oilseed rape conceptual modeling framework, constituted of root and shoot compartments. State variables describing the plant system are represented within boxes. The relationships between the state variables are presented by arrows, defined by model parameters corresponding to efficiencies or partitioning. The tested relationships are depicted in black. The total plant N quantity (QNP) results from fine root uptake (1) and N reserve remobilization (2), and determines plant area (4) after satisfying root N demand (3). Total plant C quantity (QCP) emerges from the photosynthesis activity of the plant green area (6) after excluding fallen leaves (5). Root biomass results from the growth allowed by internal N and C available quantities (7) after satisfaction of the C demand of shoots (QCS), where QCS is partitioned between stems and green leaves (8). Root biomass is then partitioned into tap roots and fine roots (9). We assumed that fine roots drive N-uptake, and N storage pool emerges when N quantities are not fully depleted by root growth and plant area establishment. N-availability is taken into account through the Specific N-uptake (SNU).

3. RESULTS

3.1. The winter oilseed rape conceptual modeling framework

From an ecophysiological point of view, the whole plant framework developed to describe winter oilseed rape response to nitrogen availability takes into account shoot and root compartments, and carbon and nitrogen fluxes, as plant structure and functions respectively. We proposed a conceptual modeling framework based on the trade-off between simplicity in terms of the number of parameters and exhaustiveness of the ARNICA model (Richard-Molard et al. 2009) in terms of oilseed rape specificities, but considering the specificity of winter oilseed rape previously described. We assessed this modeling framework for three following developmental phases: rosette growth (up to BBCH 32), stem elongation (BBCH 32 up to BBCH 59), and the reproductive phase (BBCH 59 up to seed maturity). These phases differed by the presence and state of the winter oilseed rape organs (Table IV. 2).

The proposed winter oilseed rape conceptual modeling framework is based on seventeen state variables linked by nine relationships, whose parameters all have a biological significance in relation to plant functioning (Figure IV.1 and section 3.1 for details). It describes the exchanges of C and N fluxes between the shoot and root compartments. Total plant N-quantity (QNP) results from soil-N available uptaken by fine roots through the specific N-uptake efficiency (SNU, N-uptake rate per g of cumulated fine root biomass) (**equation 1**) and from N reserve remobilization (**equation 2**). This N quantity is primarily affected to the root parts, according to the root N demand (QNR), the remaining N-quantity being allocated to the shoot (QNS) (**equation 3**). The QNS is then converted into plant area (PA) through a N-conversion-into-area efficiency (**equation 4**). Photosynthetic surface-loss are taken into account through the relationship linking fallen leaves area and PA (**equation 5**). The total plant C-quantity (QCP) results from photosynthesis activity, through the Specific C-assimilation efficiency (SCA, g of C-produced per cm² of green plant area) (**equation 6**). This parameter is estimated as the ratio between C accumulated in the whole plant and the cumulative green plant area (leaves, main stem and pods, when present). The QCP is primarily affected to the shoot parts, according to the shoot C demand (QCS), remaining C-quantity being allocated to the roots (QCR) (**equation 7**). In the shoots, carbon is partitioned between leaves and stems (**equation 8**).

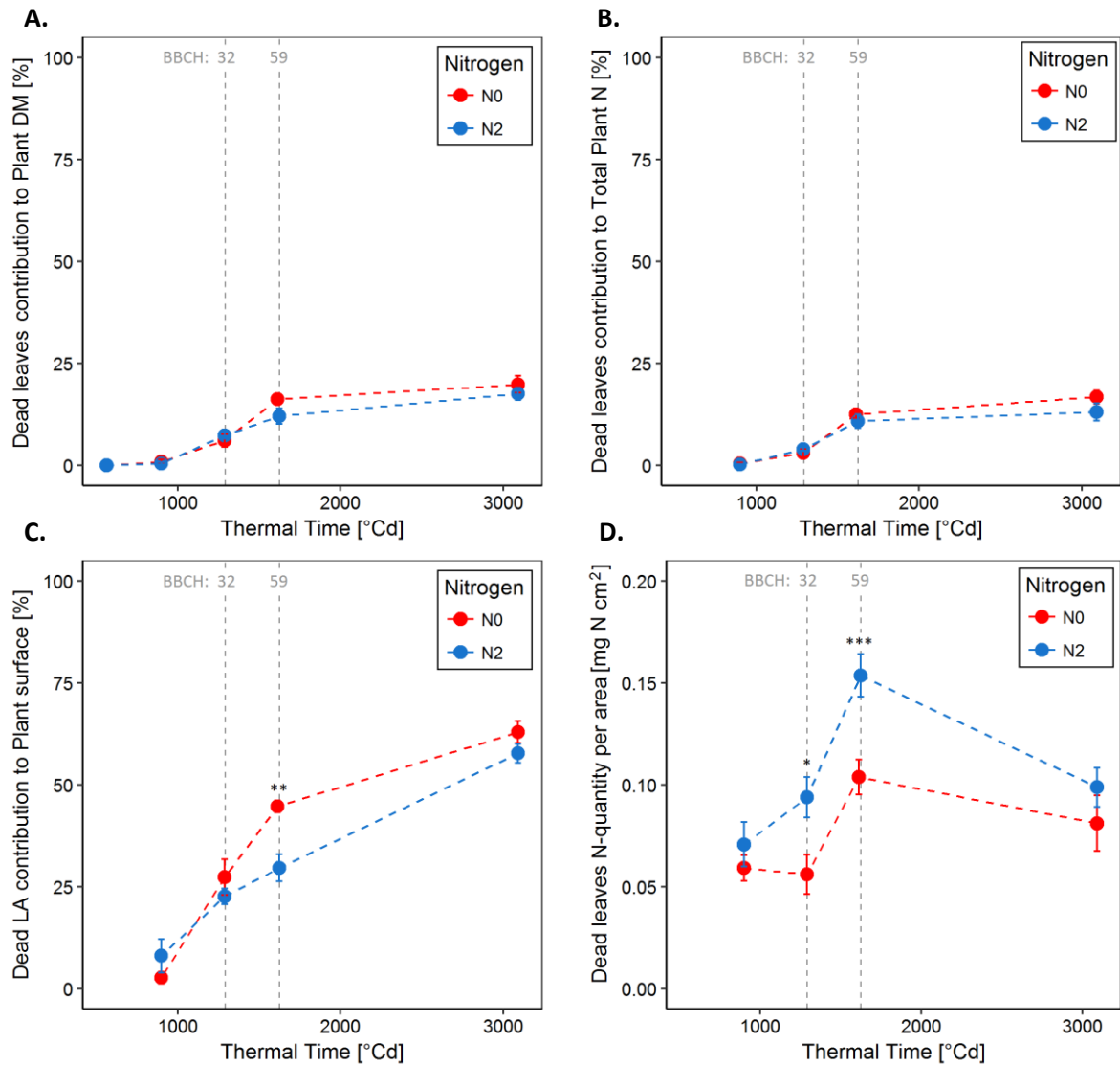


Figure IV.2. Dynamic contribution of fallen leaves to the total plant regarding A) biomass, B) N-quantity, C) area, and D) ratio of N-quantity of fallen leaves per unit of leaf area for AVISO grown under low and high N-conditions (GR18 experiment). Colors correspond to N-conditions (blue, high-N; red, low-N). Dashed lines join the plant's destructive measurements. Vertical grey dashed lines depict the beginning of stem elongation (BBCH 32) after the overwintering period and the end of vegetative growth (BBCH 59). Plant area corresponds to green leaves, fallen leaves, main stem, and pods when present on the plant. Error bars indicate the standard error of the mean. The number of stars indicates the significance level of the N-effect on the measured variable, and the absence of stars indicates non-significance: *** P-value<0.001, ** P-value<0.01, * P-value<0.05

Therefore, root growth resulted from the N quantity allocated to roots (QNR) and from the C-quantity remaining after satisfaction of shoot carbon demand (QCS). Root biomass (RDM) is partitioned between taproots (TapRDM) and fine roots (FineRDM) (*equation 9*), which drive N-uptake, in accordance with our working hypothesis. Nitrogen and carbon storage pools emerged when N and C internal quantities were not fully depleted by growth.

3.2. Quantitative analysis of the CN dynamics of fallen leaves, main stem and fine root system

The proposed model introduced three new compartments (fallen leaves, stems, and fine roots), which were not taken into account in the ARNICA model. It was thus necessary to quantify their relative contribution to the total plant biomass, area, and N amount during the whole crop cycle, and to assess the relevance to take them into account in the model. Very few studies quantified them for winter oilseed rape, especially at the individual plant level.

a). Fallen leaf area should not be neglected.

The relative contribution of the fallen leaves to the total plant biomass (Figure IV.2A), N-quantity (Figure IV.2B) and area (Figure IV.2C) increased during the growing cycle. During the rosette growth (up to BBCH 32), fallen leaves accounted for less than 7% of the total plant biomass and N-quantity but reached up to 25.5% of plant area losses, whatever the N-condition. However, fallen leaf N-quantity per cm² was significantly higher under high-N conditions (0.09 mgN cm⁻² under high-N condition compared to 0.05 mgN cm⁻² under low-N condition) (Figure IV.2D). During stem elongation and up to the end of vegetative growth (BBCH 59), these contributions increased to 11.6% for biomass and 14.1% for N-quantity, whatever the N-condition. However, fallen leaves contribution to total plant area differed significantly between N-conditions (29.6% and 44.7% under high and low-N, respectively), as well as for N-quantity per cm² (0.15 and 0.1 mgN cm⁻² at high and low-N, respectively). During the reproductive period, fallen leaves reached their maximum contribution, accounting for up to 18.7% of total plant biomass, 19.1% of total N-uptake, and 60.3% of the total plant area, and did not significantly vary between N-conditions.

Linear regressions linking biomasses or areas of fallen and green leaves were assessed using data from the GR18 experiment from BBCH 19 to BBCH 59 (Supplementary figure IV.S2). The relationship between dry matters was not significant under low N-conditions ($R^2= 0.24$, $P\text{-value}= 0.91$) and was significant under high N-conditions ($R^2= 0.78$, $P\text{-value}=0.04$), suggesting that the green leaf biomass was not a pertinent trait to estimate fallen leaf biomass. In contrast, we observed a significant linear relationship between the fallen leaf area and the green plant area up to BBCH 59, with a strong significant impact of the N-condition on the slopes ($P\text{-value}= 3.01 \cdot 10^{-5}$). As we did not measure the fallen leaf areas in the GR15 experiment, we estimated it from the measured green plant areas by using the relationship established on the GR18 experiment data. We therefore highlighted that fallen leaves should not be neglected, especially in terms of surfaces and under low-N conditions.

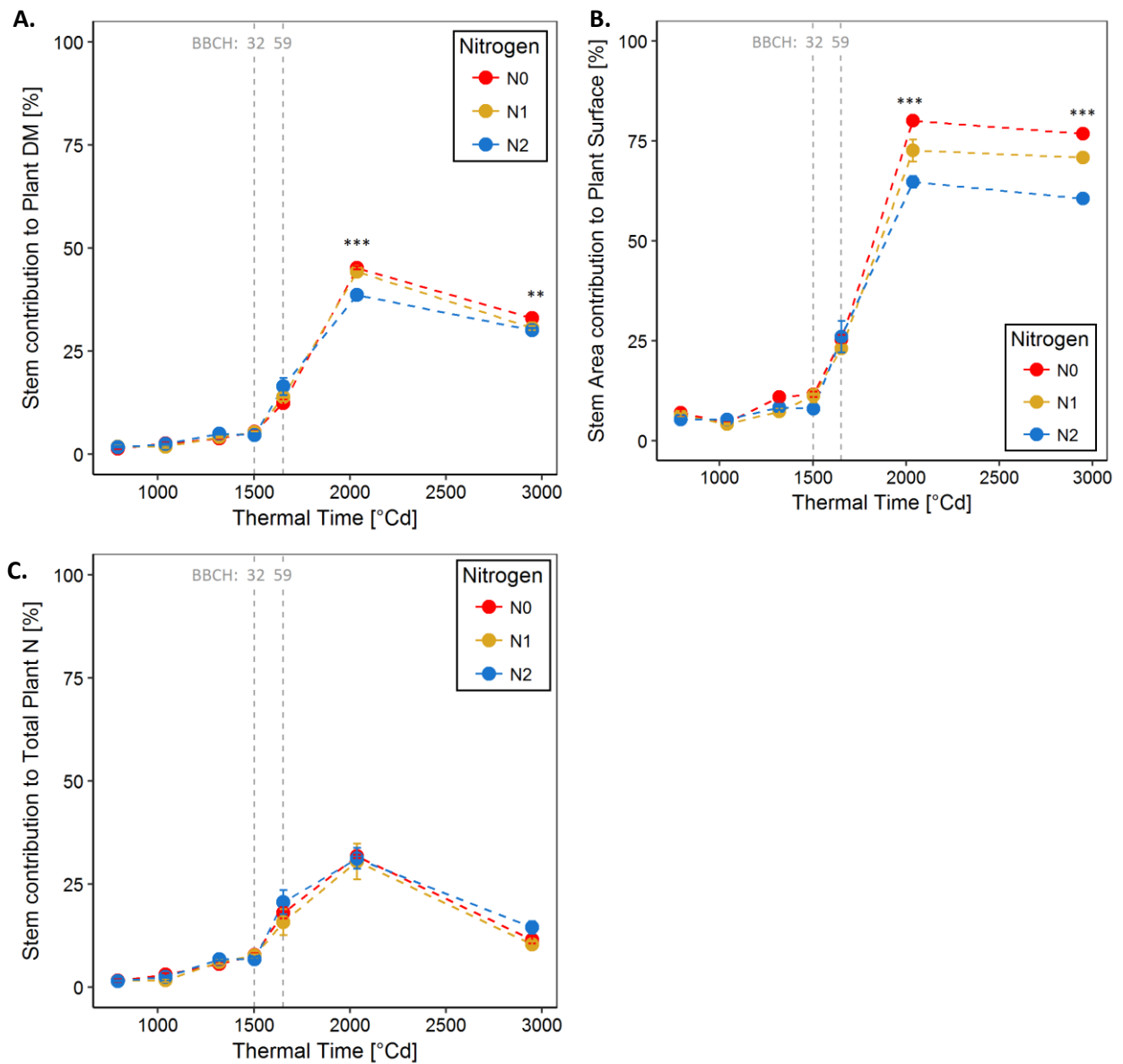


Figure IV.3. Dynamic contribution of the stems to total plant dry matter (A), total plant area (B) and total plant N-quantity (C) for AVISO grown under three contrasting N-conditions in the GR15 experiment. Stems included the main stem and branches for biomass and N-quantity, but only the main stem for area measurements. Colors correspond to N-conditions (blue, high-N; yellow, medium-N; red, low-N). Dashed lines join the plant destructive measurements. Vertical grey dashed lines depict the beginning of stem elongation (BBCH 32) after the overwintering period and the end of vegetative growth (BBCH 59). Error bars indicate the standard error of the mean. The number of stars indicates the significance level of the N-effect on the measured variable, and the absence of stars indicates non-significance: *** P-value<0.001, ** P-value<0.01, * P-value<0.05

b). Stems should not be neglected during the reproductive phase

We studied the dynamic contribution of the stems to the whole plant biomass, N-quantity and area through the crop cycle (Figure IV.3). Up to BBCH 32, only a small part of the main stem is developed and contributed less than 5% to total plant biomass and less than 7% to the total plant N-quantity, regardless of N-condition. Main stem elongation started after the overwintering period (BBCH 32), to reach its maximum length at the end of vegetative growth just before flowering (BBCH 59). At BBCH 59, the main stem accounted for up to 14% of the total biomass, 25% of the total plant area and 19% of the total plant N-quantity, regardless of N-condition.

During flowering period, lateral branches quickly elongated, increasing the contribution of the stem compartment to whole plant biomass and N quantity, but also green area, Although we measured branches biomass and N-quantity, we did not measure branches area in our experiments. Thus the contribution of stems to plant green area was underestimated in our results. Nevertheless, stems (main stem and branches) contribution to total plant biomass, and N-quantity linearly increased until the end of flowering, with a significant effect between N-treatments ($P\text{-value} = 2.18 \cdot 10^{-5}$), except for the N-quantity. After flowering (BBCH 71), stems accounted for up to 45.2% of total plant dry matter at low-N and 38.6% at high-N conditions. Stems contribution to the total plant N-quantity, reached a maximum of 31.4% at the end of flowering (corresponding to $0.2 \text{ gN plant}^{-1}$), decreasing to 12.9% at harvest (corresponding to $0.1 \text{ gN plant}^{-1}$).

These results led us to differentiate three phases of stem contribution during the plant development in the winter oilseed rape conceptual modeling framework. The first phase up to BBCH 30-32, corresponds to a very low contribution of stems, after which the contribution increased linearly up to BBCH 71 to finally reach a plateau or decreasing up to harvest.

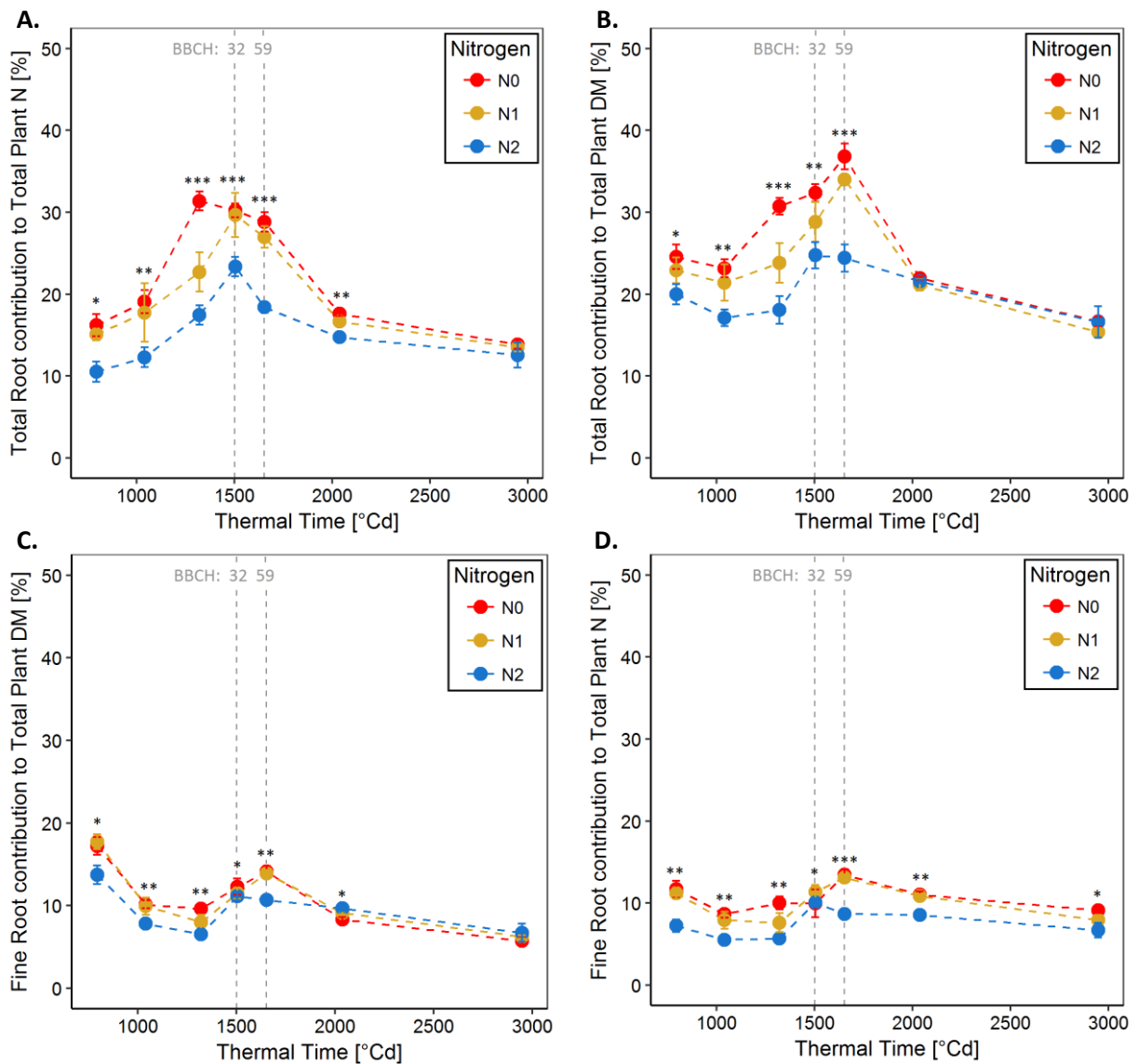


Figure IV.4. Dynamic contribution of the total root system (fine + tap roots) to total plant dry matter (A), plant N-quantity (C); and contribution to fine roots to total plant biomass (C), plant N-quantity (D) for AVISO grown under three N-conditions in the GR15 experiment. Colors correspond to N-conditions (blue, high-N; yellow, medium-N; red, low-N). Dashed lines join the plant destructive measurements. Vertical grey dashed lines depict the beginning of stem elongation (BBCH 32) after the overwintering period and the end of vegetative growth (BBCH 59). Error bars indicate the standard error of the mean. The number of stars indicates the significance level of the N-effect on the measured variable, and the absence of stars indicates non-significance: *** P-value<0.001, ** P-value<0.01, * P-value<0.05

c). The root system: highlighting fine roots contribution

Winter oilseed rape root system is characterized by taproots, which act as a reservoir for nutrients and assimilates, unlike fine roots that support water and nutrient uptake and present the ability to branch out and proliferate in zones of higher nutrient content. Therefore, to assess the C and N fluxes, the whole root system should be considered, and each of its two compartments quantified (Figure IV.4).

The contribution of the root system to the total plant dry matter increased throughout the vegetative growth, reaching a maximum before flowering. It was significantly affected by N-conditions (P -value = $4.42 \cdot 10^{-4}$). We observed that root contribution to total plant DM was always higher under low-N than under high-N conditions. At BBCH 59, we report a contribution of the root system to whole plant biomass of 36.8% under high-N and 24.4% under low-N conditions, respectively. During flowering, the root contribution to plant dry matter sharply decreased under the low-N condition, compared to the high-N condition, which showed a less pronounced decrease. After flowering, no significant differences between N-conditions were observed. The contribution of roots to plant N-quantity reached a maximum at BBCH 32 (31.4% and 23.4 % under low-N and high-N conditions, respectively), and was reduced during the stem elongation period regardless of N conditions. At the end of flowering, roots still accounted for 17.6% and 14.8 of the whole plant N-quantity under low-N and high-N conditions respectively.

Fine roots reached a maximum contribution to total plant biomass and total plant N quantity at the early stages and before flowering (BBCH 59), accounting for up to 14.1% of the total plant biomass under the low-N condition and 10.6% under the high-N condition (P -value= 0.003). They accounted for up to 13.4% of the total N-quantity under low-N and high-N conditions, respectively (P -value= $6.09 \cdot 10^{-4}$). We therefore highlighted that fine roots represented an important compartment for biomass and N-quantity, hence they were not negligible in winter oilseed rape.

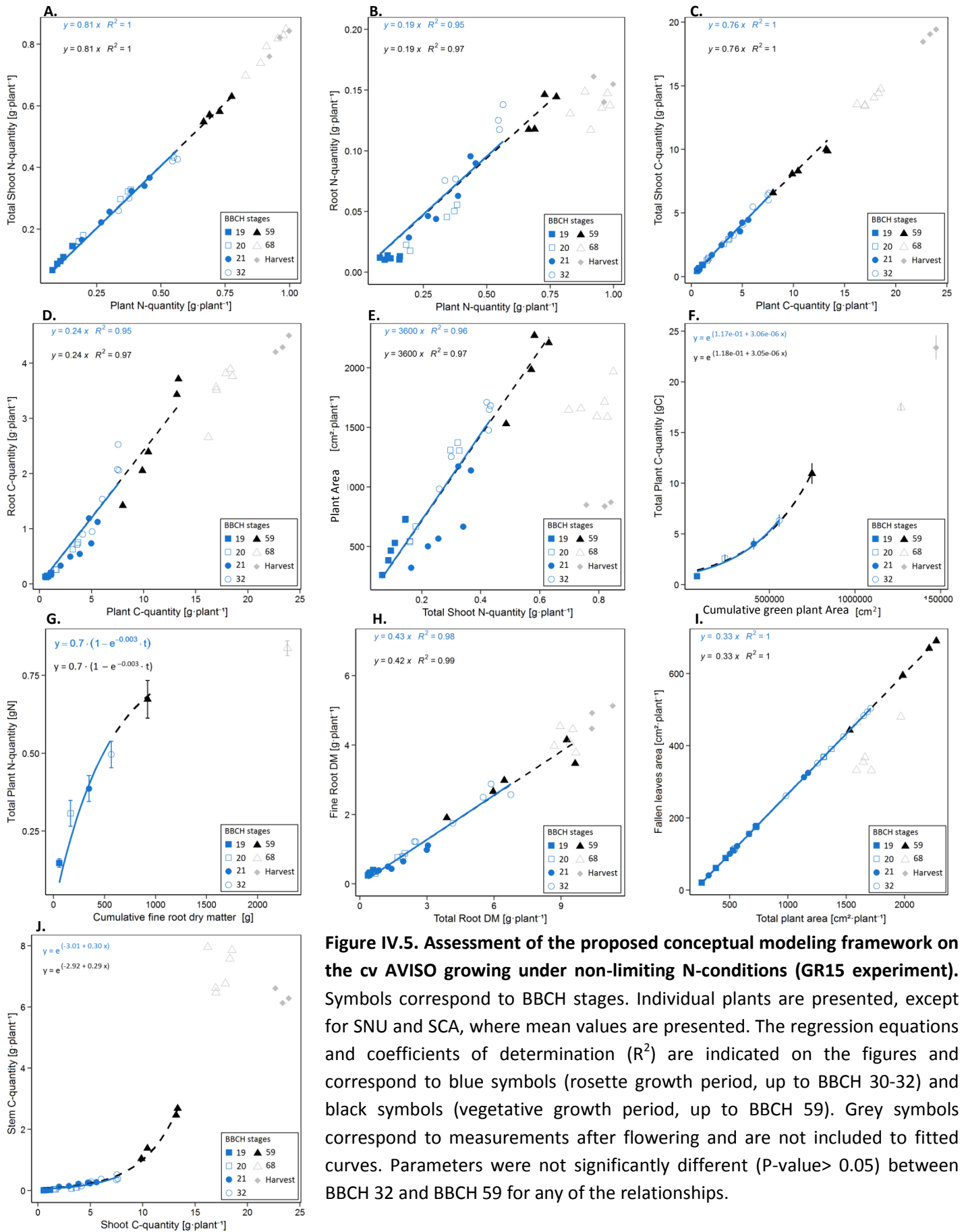


Figure IV.5. Assessment of the proposed conceptual modeling framework on the cv AVISO growing under non-limiting N-conditions (GR15 experiment). Symbols correspond to BBCH stages. Individual plants are presented, except for SNU and SCA, where mean values are presented. The regression equations and coefficients of determination (R^2) are indicated on the figures and correspond to blue symbols (rosette growth period, up to BBCH 30-32) and black symbols (vegetative growth period, up to BBCH 59). Grey symbols correspond to measurements after flowering and are not included to fitted curves. Parameters were not significantly different (P -value > 0.05) between BBCH 32 and BBCH 59 for any of the relationships.

3.3. Estimation of the model parameters during rosette growth period

We first established relationships for each equation of the conceptual modeling framework under the non-limiting N-condition and for cv AVISO, using the data of the GR15 experiment for the growing period lasting from sowing to the beginning of stem elongation (BBCH 30-32). All plant compartments, including fine roots and fallen leaves, were taken into account for C and N amount calculations, to accurately establish N and C fluxes. Some relationships could not be assessed during this work, because the data collected were not suitable for this purpose. Particularly, the C and N storage and remobilization cycle was not evaluated (**Figure IV.1, relationship 2**).

The specific N-uptake efficiency (SNU) (**Figure IV.1, relationship 1**) was assessed by the N-quantity taken up per g of cumulative fine root biomass (Figure IV.5G). Due to the absence of sampling dates between flowering and harvest, we could only calculate the SNU parameter up to late flowering. A negative exponential function was fitted between the cumulative fine root biomass and the total plant N quantity from sowing to BBCH 30-32 ($y=0.7[1-\exp(0.003x)]$, RMSE=0.01). Thus, SNU tended to decrease with time.

Regarding the partitioning of the total plant N- amounts (**Figure IV.1, relationship 3**) between shoot and root compartments, we found significant linear relationships with high coefficients of determination between the total plant N-amount and N-amounts in the shoot ($R^2=1$, RMSE= 0.02 Figure IV.5A) and in the roots ($R^2= 0.95$, RMSE=0.02 Figure IV.5B). Thus, 81% of total nitrogen quantity in the plant was allocated to shoots and 19% to roots during the rosette growth phase. We observed the same pattern and ratio for the partitioning of the total C-quantity into shoots and root compartments (Figure IV.5C and IV.5D) (**Figure IV.1, relationship 7**).

The conversion efficiency of nitrogen into plant area (**Figure IV.1, relationship 4**) was assessed by considering only the green and fallen leaf area per g of total shoot N-quantity, as the stems were neglectable at this stage (cf. 3.2). We found a significant linear regression linking these two state variables ($R^2= 0.96$, RMSE= 136.1 Figure IV.5E). We accounted for leaf area losses by showing that the fallen leaves area was related to the total leaf area (**Figure IV.1, relationship 5**) by a strong linear relationship ($R^2= 1$, RMSE= 53.55 Figure IV.5I).

The specific C-assimilation efficiency (SCA) (**Figure IV.1, relationship 6**) was estimated from the cumulative green plant area (GPA), as we showed that leaves were the main contributors to photosynthetic plant area at this stage. Total plant C quantity and cumulative green plant area were linked by an exponential function ($y=\exp[1.17 \cdot 10^{-1} + 3.06 \cdot 10^{-6} x]$, RMSE= 1.64), whose two parameters correspond to SCA (Figure IV.5F). We noticed that SCA was not constant but increased during the rosette growth. Regarding the partitioning of the shoot C-amount into stem versus leaf compartments (**Figure IV.1, relationship 8**), we found that the part of C allocated to the stems followed an exponential function ($y=\exp[-3.01+0.30x]$, RMSE=0.24) (Figure IV.5J).

Interestingly, we noticed that the partitioning of the total root biomass between tap and fine roots (**Figure IV.1, relationship 9**) was constant during the rosette period ($R^2= 0.96$, RMSE= 0.2 Figure IV.5H), with 43% of the root biomass partitioned to fine roots and 57% to taproots. This result was confirmed on the GR18 experiment, where the biomass ratio of fine root to total roots was 38% for AVISO under the high-N condition. (Table IV. 4).

3.4. Validity to the conceptual framework during the whole vegetative growth

As a second step, we assessed the validity of the established relationships up to the end of vegetative growth, just before flowering (BBCH 59). This second step includes the development of the main stem from BBCH 30-32, which thus account for a non-longer negligible part of the photosynthetic area and of the C and N quantities of whole plant (Figure IV.3). For that reason, the plant compartments considered during this period included tap and fine roots, green and fallen leaves and the main stem. Ramification stems were neglected at this stage.

We found that the relationships established during rosette growth were conserved until the end of vegetative growth (BBCH 59) (Figure IV.5, black symbols, curves and regressions). Indeed, we did not find any significant differences (P-values > 0.05) between the parameters established during rosette and vegetative growth (Figure IV. 5). Thus, taking into account the C-N quantities and areas of the main stem, in addition to the other plant compartments already considered up to BBCH 30-32, we were able to describe C-assimilation, N-uptake, and C-N partitioning up to BBCH 59.

3.5. Could we extend the conceptual modeling framework up to the seed filling period?

We investigated if the relationships validated up to the end of the vegetative growth might be extended to the reproductive period. We therefore included pod contribution to C-N quantities and photosynthetic areas as well as ramification stems contribution to C-N quantities.

The equation adjusted for the partitioning of the total root biomass into tap and fine roots (Figure IV.5H) remained valid up after flowering, with no significant difference on the value of the parameters. However, parameters defining the other relationships were significantly different between vegetative and reproductive growing periods.

Therefore, the proposed equations for modeling winter oilseed rape C and N fluxes were validated up to the end of the vegetative growth, but not later. This result pointed out a change in plant functioning and related processes, especially concerning radiation assimilation efficiencies, conversion of N into areas, and the allocation of C and N assimilates between the shoot and root compartments.

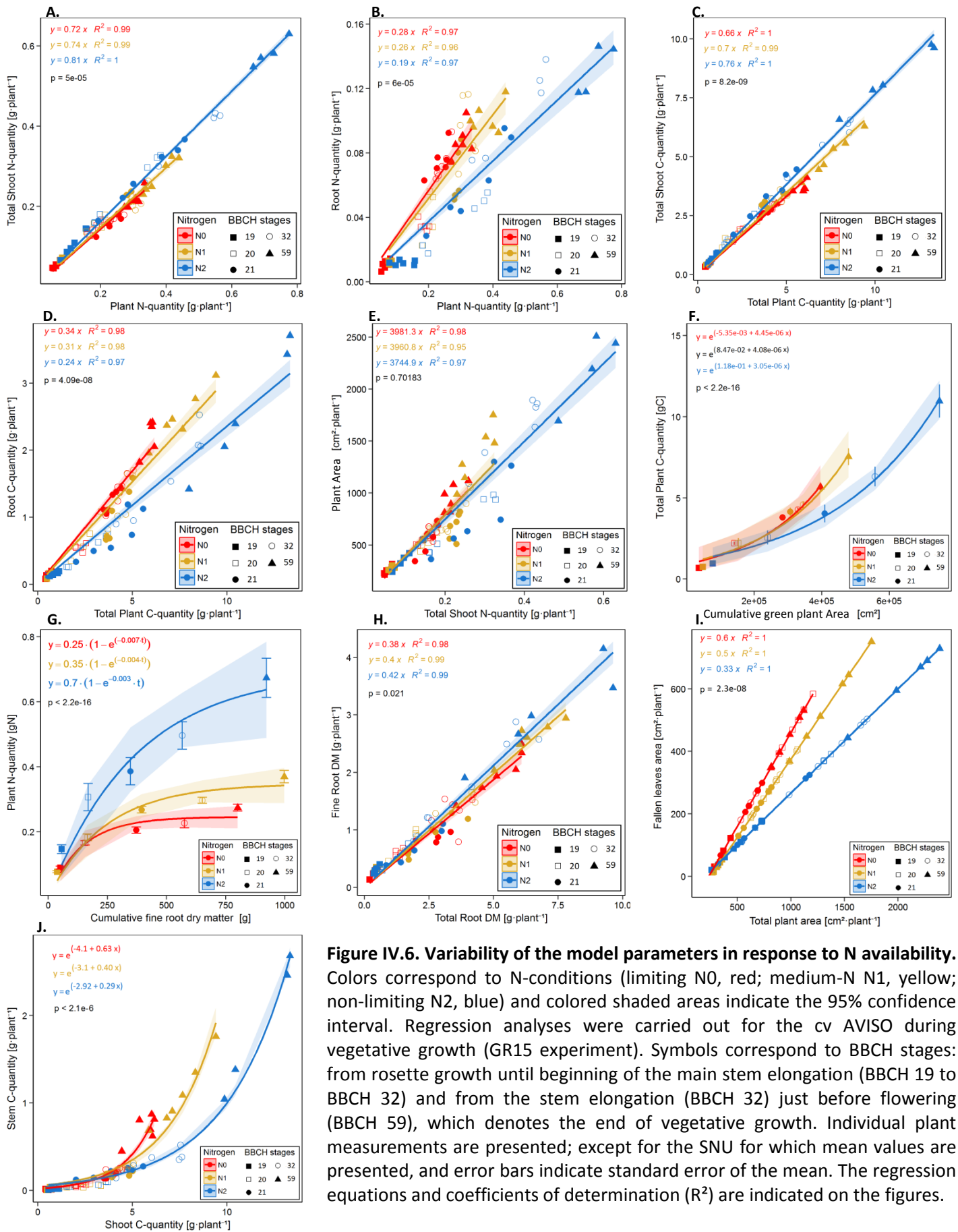


Figure IV.6. Variability of the model parameters in response to N availability. Colors correspond to N-conditions (limiting N0, red; medium-N N1, yellow; non-limiting N2, blue) and colored shaded areas indicate the 95% confidence interval. Regression analyses were carried out for the cv AVISO during vegetative growth (GR15 experiment). Symbols correspond to BBCH stages: from rosette growth until beginning of the main stem elongation (BBCH 19 to BBCH 32) and from the stem elongation (BBCH 32) just before flowering (BBCH 59), which denotes the end of vegetative growth. Individual plant measurements are presented; except for the SNU for which mean values are presented, and error bars indicate standard error of the mean. The regression equations and coefficients of determination (R^2) are indicated on the figures.

3.6. How did the model parameters vary according to N-condition?

We used the proposed conceptual modeling framework to identify and hierarchize the main processes and parameters describing oilseed rape responses to N availability up to flowering. We investigated the variation of model parameters during vegetative growth for the cv AVISO grown under low (N0), medium (N1), and high (N2) nitrogen conditions in the GR15 experiment (Figure IV.6). We confirmed the results on data obtained on cv AVISO in the GR18 experiment under low-N and high-N conditions (Table IV.4).

As a first step, ANOVAs were performed at each sampling date to test the N effect on the state variables of the modeling framework, by using the data of the GR15 and the GR18 experiments on the cv AVISO (Supplementary IV.S1). As expected, state variables were highly sensitive to N-conditions, except for nitrogen and carbon quantities in roots at early beginning of growth (from sowing up to BBCH 32).

As a second step, we evaluated the effect of N-condition on the model parameters. Most of the parameters varied in response to N-condition, except for two: conversion efficiency of N into plant area (PS/QNS) and fine root contribution to the whole-root system (FineRoot/RDM) (Figure IV.6 and Table IV.4). There was a significant effect of the N-condition on N-uptake efficiency and partitioning. The specific N-uptake (SNU) was higher under high-N conditions compared to low-N conditions: 1 g of cumulated fine roots absorbed 0.21 mg of N under high-N conditions vs 0.17 mg of N under low-N conditions. Regarding the allocation of the total N-amount into the shoot and root compartment, under low-N there was an increase of 10% of N allocated to the roots (Figure IV.6 A and B). This partitioning pattern was also observed on carbon, with a significant increase in the carbon allocated to roots under limited N-supply (Figure IV.6 C and D).

In order to hierarchize the impact of N on the various model parameters, we estimated the absolute plasticity of each parameter by considering the difference between non-limiting and limiting N-conditions (Figure IV.7A). We distinguished three groups of parameters, displaying either high (>60%), medium (20-60%), or low (<20%) plasticity. The relative contribution of fallen leaves to total plant area (FLA/PA), the specific C-assimilation (SCA), and the specific N-uptake efficiency (SNU) were the most plastic parameters (~80%) supporting the main part of the response of winter oilseed rape to nitrogen nutrition, followed by the parameters driving CN allocation to the roots (QNR/QNP and QCR/QCP) and the allocation of carbon in shoots to stems (~40%). Globally, the allocation parameters of the total CN amount to total shoot dry matter showed low absolute plasticity (QNS/QNP and QCS/QCP, <10%). Parameters regarding the conversion efficiency of the shoot N-amount into plant area (PA/QNS) and the fine roots ratio (FineR/RDM) showed low (10%) or nearly no plasticity (<5%).

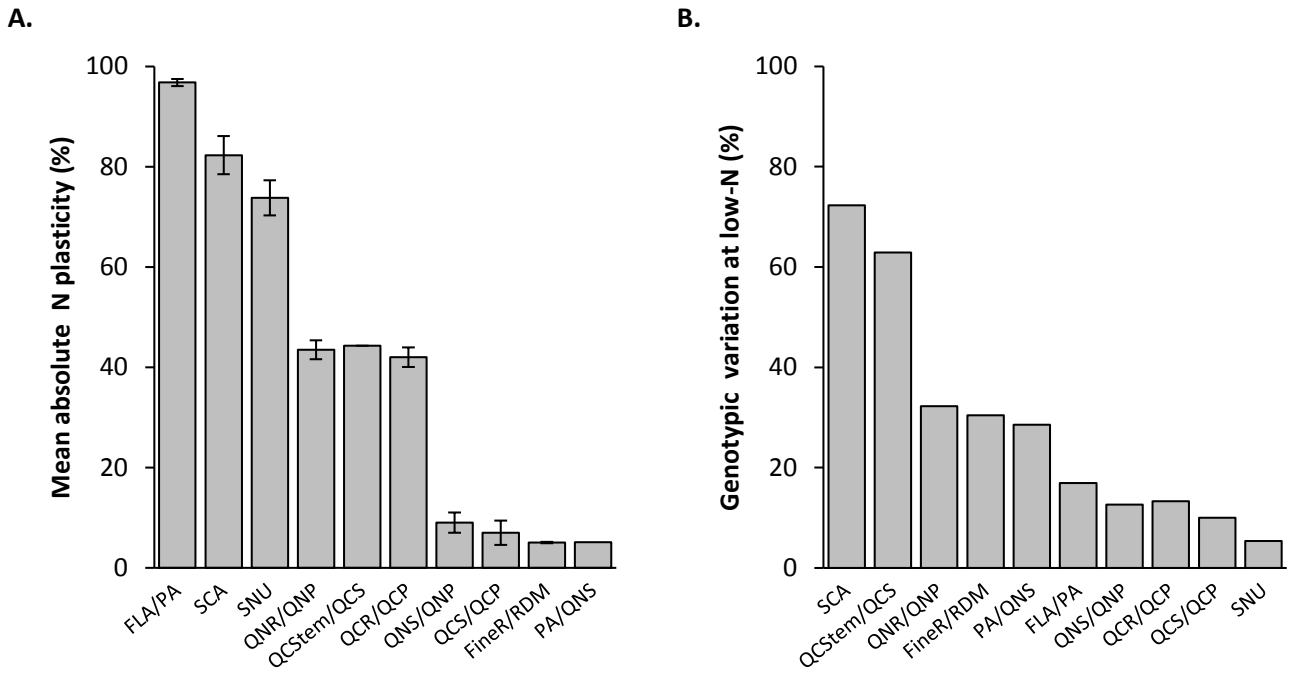


Figure IV.7. Mean absolute plasticity in response to N-condition (A) and genotypic variation (B) for eight allocation/efficiency model parameters. Mean N plasticity refers to AVISO's ability to exhibit different parameters under low-N (N0) versus high-N (N2) conditions, using GR15 and GR18 datasets. Error bars depict standard errors of the mean and have been calculated only for N-plasticity. Genotypic variation corresponds to the difference between the highest and the lowest parameter value and the maximal value observed across the five genotypes grown under the low N-condition in the GR18 experiment. Parameters are defined in Table IV.3.

3.7. How did the model parameters vary with genotype under the low-N condition ?

The studied parameters were highly dependent on the N-condition (Figure IV.6 and IV.7). Besides in this study we did not account for the GxN, we assumed that the state variables used to calculate the investigated parameters might exhibit GxN interaction as it has been reported in the literature. Therefore, we evaluated the genotype effect on the parameters under low-N supply and during vegetative growth, only using the GR18 data set (Table IV.4). All the state variables considered showed a significant genotypic effect (Supplementary Table IV.S1),

We showed a significant genotype effect on the model parameters (Table IV.4.), but genotypes did not statistically differ in specific N-uptake (SNU; maximum P-value=0.96). Contrasting trends were found among the genotypes on the CN allocation parameters, as EXPRESS allocated 67% of the total N-amount to the shoot compartment while AVISO allocated up to 79%. The same pattern was observed for the allocation of the total C-amount into the shoot and root compartment but with fewer (up to 2%) but significant (P-value < 0.01) differences between genotypes. Interestingly, genotypes also exhibited contrasting efficiency in the conversion of shoot N-amount into plant area, with OLESKI and EXPRESS exhibiting the lowest efficiencies, and AMBER, AVISO, and MOHICAN exhibiting the highest efficiencies.

In order to hierarchize the relative genotypic variation of the model parameters, we estimated the genotype variation of each parameter by considering the difference between the highest and lowest parameter value among the genotypes (Figure IV.7B). SCA and the C partitioning into stems (QCStem/QCS; Figure IV.1 equation 8) exhibited the highest variation among genotypes (up to 72%). Medium but still significant genotypic variation (~30%) was observed for the allocation of the whole plant nitrogen to roots (QNR/QNP; Figure IV.1 equation 3) as well as for the conversion of the total shoot N-amount into plant area (PA/QNS) and the partitioning of the whole root system into fine roots (FineR/RDM; Figure IV.1 equation 9). Lower genotypic variation (from 10 to 20%) was found for the fallen leaves area ratio (FLA/PA; Figure IV.1 equation 5), the allocation of the whole plant N-amount to the shoot compartment (QNS/QNP), the allocation of the whole plant C-amount to roots and shoots, respectively.

These results highlighted that the main source of genotypic variation during the vegetative growth under the low-N condition relied first on specific carbon assimilation (SCA) and carbon partitioning between leaves and stems, and second on N fluxes between the root and shoot compartment, the conversion of shoot N-amount into leaf area and the fine root ratio.

Table IV.4. Variation of the parameter values according to N-condition and genotype in the GR18 experiment. N effect was evaluated on the AVISO genotype grown under both low and high N-conditions, whereas the genotypic effect was tested on five winter oilseed rape genotypes (AVISO, AMBER, EXPRESS, MOHICAN, and OLESKI) grown under the low-N condition only. The difference in significance of parameter values according to genotype (G) and nitrogen condition (N) was tested. Significant codes include *** p value<0.001, ** 0.01<p value<0.001, * 0.05<p value. NS=non-significant.

Equation/Parameter	Corresponding equation Fig. IV.1.	GR18-N2 GR18-N0						N	G
		Aviso	Aviso	Amber	Express	Mohican	Oleski		
QNP=a[1-exp(-b·FineRDM)]	Eq. 1								
SNU_a		1.34	0.30	0.28	0.27	0.29	0.28	***	ns
SNU_b		9.1E-04	4.2E-03	6.7E-03	5.5E-03	4.9E-03	6.4E-03	***	ns
QNR = a(QNP)	Eq. 3a								
QNR/QNP		0.15	0.21	0.25	0.33	0.24	0.27	*	***
QNS=a(QNP)	Eq. 3b								
QNS/QNP		0.85	0.79	0.75	0.67	0.76	0.73	***	***
QCR=a(QCP)	Eq. 7a								
QCR/QCP		0.14	0.18	0.19	0.20	0.18	0.20	***	**
QCS=a(QCP)	Eq. 7b								
QCS/QCP		0.86	0.82	0.81	0.80	0.82	0.80	***	**
PA=a(QNS)	Eq. 4								
PA/QNS		1918.4	1941.0	1978.5	1788.7	1818.9	1448.8	ns	**
QCP=exp(a+b·PA)	Eq. 6								
SCA_a		6.7E-01	4.4E-02	6.2E-02	-4.8E-01	3.0E-03	1.1E-01	***	***
SCA_b		4.0E-06	7.1E-06	7.6E-06	1.4E-05	7.9E-06	7.9E-06	***	***
FineRDM=a(RDM)	Eq. 9								
FineR/RDM		0.38	0.37	0.38	0.46	0.39	0.32	ns	***
FLA=a(PA)	Eq. 5								
FLA/PA		0.33	0.63	0.56	0.64	0.67	0.64	***	***
QCStem=exp(a+b·QCS)	Eq. 8								
QCStem/QCS_a		-2.92	-1.28	-0.73	-3.25	-2.00	-2.50	***	***
QCStem/QCS_b		0.29	0.42	0.34	0.90	0.52	0.72	***	***

4. DISCUSSION

4.1. Why is it important to consider parameters instead of state variables to understand N-use efficiency?

N-availability affects C and N assimilation and partitioning between roots and shoots (reviewed in Lammerts van Bueren et al., 2017). In this study, we observed contrasting plant growth between low and high N-conditions, with significant genotypic variation in shoot and root biomass under the low-N condition, as soon as rosette development began. As it has already been reported in oilseed rape (Svečnjak and Rengel, 2006a,b; Ulas et al., 2013; Stahl et al., 2015; Wang et al., 2017), we noticed that environmental conditions and genotype significantly impacted the integrative state variables underlying NUE and reflecting plant growth (e.g. shoot and root dry matter, total N amount, and photosynthetic plant areas). Moreover, for a given state variable, nitrogen and genotypic impacts also varied according to the plant's growth phase, which complexify the identification of appropriate target traits for characterizing these dynamic processes. This was also reported by Lievre et al. (2013), who showed that plants with rapid leaf expansion in early development stages did not necessarily exhibit larger final leaf area. Thus, the final phenotype could not necessarily be represented by an early punctual measurement of a dynamic process.

Similarly, Granier et al. (2014) reported that the observed phenotype at a given stage could not be restricted to one trait characterizing a genotype. Instead, the authors suggested disentangling individual traits in a network by defining the relationships between them via model parameters. Parameters can be considered as efficiencies, whereas integrative variables are the consequences of these efficiencies (Martre et al., 2015). In this study, we highlighted specific N-uptake (SNU), carbon assimilation efficiency (SCA), and fallen leaves area ratio (FLA/PA) as the key traits explaining the plant's response to N-availability. Moreover, we found that SCA and the stem C-allocation ratio (QCStem/QCS) were also the key traits explaining the observed genotypic variation under low-N availability.

4.2. A first conceptual modeling framework to unravel winter oilseed rape vegetative growth

Several ecophysiological plant-models exist for oilseed rape, such as a functional-structural model describing yield elaboration from the end of the rosette period (Groer et al., 2007), an architectural shoot model simulating the dynamics of organ growth from sowing to harvest (Jullien et al., 2007; Jullien et al., 2011), and a FSPM focusing on the growth of oilseed seedling leaves during autumn (Tian et al., 2017). Other oilseed rape crop models mainly focus on accurate descriptions of the rates of photosynthesis, respiration, and biomass production of leaves and pods at the crop scale (Müller et al., 2005); the N partitioning between vegetative and reproductive tissues from stem elongation to harvest (Malagoli et al., 2005a); sulfur uptake, allocation and remobilization during vegetative growth until the onset of pod formation (Brunel-Muguet et al., 2015); and crop growth, development and yield under different environmental conditions and N treatment (Böttcher et al. 2000).

The first outcome of this study is a conceptual modeling framework describing winter oilseed rape whole-plant C-N functioning and characterizing the dynamic processes of biomass accumulation and C and N fluxes in response to N-conditions, at the whole plant level. Interestingly, all the parameters used in the proposed modeling framework might be measured under controlled or semi-controlled field-like conditions. A part of this framework was drawn from the whole-plant model ARNICA (Richard-Molard et al., 2009), dedicated to *Arabidopsis thaliana*, but it had to be extended to take into account winter oilseed rape specificities, and adding the pools of fallen leaves and stems and by splitting the root pool into fine roots and taproots.

To define this conceptual modeling framework, we made several assumptions regarding N-uptake (1), conversion of C captured by the plant area into biomass (expressed as C-quantity) (2), and contribution of fallen leaves to plant functioning (3).

1). Although the whole root system was considered for the quantifications of C and N fluxes, we hypothesized that only fine roots drove N uptake. This assumption has already been supported in winter oilseed rape by Malagoli et al. (2004), because taproot represents less than 9% of total N-absorption just before flowering (Rossato et al., 2001). Moreover, although SNU is usually defined per unit of cumulated root length, we approximated fine root length by fine root biomass because the total root length was not measurable in our culture device. This variable is indeed difficult to measure in complex root systems, especially for old plants, grown under field like-conditions (Ulas et al., 2013). Besides, we hypothesized that the N-uptake rate mainly depended on the cumulative fine root biomass and that N-uptake was uniformly distributed within the root system. The same hypotheses on SNU were also assumed in legumes by Moreau et al. (2012b). However, N uptake seemed more related to root length, which determines root exchange area, rather than to root biomass in wheat (Laperche et al., 2006), because this biomass can be invested in root architecture in various ways depending on growth conditions and genotype (Garnett et al., 2009; Ulas et al., 2012; He et al., 2017; Wang et al., 2017). In our study, SNU was fitted by a negative exponential function from sowing to the end of flowering, which was in opposition to the linear adjustment described in wheat (Laperche et al., 2006) and *Arabidopsis thaliana* (Richard-Molard et al., 2008). The different adjustments might be explained by i) the dynamic variations on N-availability under field-like conditions compared to constant N supplies under controlled N-conditions, ii) the ontogenetic reduction of root growth during the plant growth-cycle and iii) root growth limitation by soil volume.

2). We approximated specific carbon assimilation efficiency (SCA) by considering whole-plant carbon amount as a function of the whole-plant area (cumulative green plant area) instead of the projected plant area. This assumption might produce two impacts on the estimation of the parameters. First, considering the total green leaves area instead of the projected leaves area might result in an overestimation of light interception by the plant within a canopy. Therefore, we referred to the plant's potential capacity to intercept radiation and convert it into biomass without considering leaf shading. Second, we assumed that pods mainly intercept incoming radiation during reproductive growth, limiting the role of branches in the light interception. Although lateral branches might participate in light interception, we did not account for their area as we did for main stem. This assumption might also impact the efficiency of shoot-N conversion into plant area after BBCH 59, and therefore, the validity range of the proposed modeling framework. Green leaves are the main organs intercepting incoming solar radiation during vegetative growth, but stems and pods are the main contributors to the total plant area during the reproductive phase. Little data on photosynthetic efficiency of pods is available, but Leterme et al. (1985) and Yu et al. (2010) showed that pod walls are photosynthetic organs. However, the maximal photosynthetic efficiency of pods is approximately two times lower than that of leaves (Gammelvind et al., 1996; Müller and Diepenbrock, 2006). Moreover, in this study, SCA was described by an exponential function, which is in line with the results reported on spring oilseed rape by Lemaire et al. (2007). However, it differs from the results found by Richard-Molard et al. (2009) on *Arabidopsis thaliana*, but their results were obtained at rosette stage under constant lighting conditions in growth chambers and referred to projected plant area.

3). Winter oilseed rape loses high quantities of N of over the crop cycle via leaf fall (Allirand et al., 2007), thereby impacting C and N fluxes. Malagoli et al. (2005a) reported that N loss by fallen leaves from stem extension to harvest time amounted to 45 kg N ha⁻¹ in the field, representing 11.6% of total N content taken up by the plant from stem extension to harvest. However, in our study, these values were slightly higher, probably because all the fallen leaves were collected exhaustively, and N-losses accounted for 99.3 kg ha⁻¹, representing 19.1% of the total N taken up by the plant from sowing to harvest. We also highlighted that fallen leaves might impact the photosynthetic area since early growing stages because leaf fall began as soon as 59 days after sowing (equivalent to 742 growing degree days). At the end of the vegetative growth, we noticed that 52% of the total leaf area produced corresponded to fallen leaves under the low-N condition and 28% under the high N-condition. However, although green leaf areas remain measurable at a given growth stage, quantifying the total produced leaf area—including the area of fallen leaves—remains tedious. We therefore proposed the green leaves area as a proxy trait to estimate fallen leaf area during vegetative growth. However, this result requires further investigation, particularly under contrasting N-conditions.

4.3. N-uptake and C-assimilation efficiencies as well as fallen leaves contribution to plant area were key parameters explaining variations in response to contrasting N-conditions

As a second main outcome, the use of the conceptual modeling framework led us to underline the role of specific C-assimilation (**SCA; Figure IV.1 equation 6**), specific N-uptake (**SNU; Figure IV.1 equation 1**), and fallen leaves contribution to plant area (**FLA/PA; Figure IV.1 equation 5**) as the main parameters underlying winter oilseed rape responses to N-conditions during the vegetative growth. Regarding parameters supporting the genotypic variations under the low-N condition, we highlighted SCA and the part of C-shoot allocated into stems (**QCStem/QCS; Figure IV.1 equation 8**) as the two main ones. Nevertheless, we highlighted the N-amount allocated to roots (**QNR/QNP; Figure IV.1 equation 3**), the conversion of the shoot N-amount into plant area (**PA/QNS; Figure IV.1 equation 4**) and the fine root ratio (**FineR/RDM; Figure IV.1 equation 9**) as important parameters describing the observed genotypic variation under the low-N condition. These observations were in opposition with the results obtained by Richard-Molard et al. (2009) on *Arabidopsis thaliana*, who reported that genotypic variation in SNU and SCA were sufficient to explain genotypic differences in response to contrasting N-conditions, and with the results of Laperche et al., (2006) on wheat, where genotypes differed in SNU. However, in our study we tested a smaller set of five genotypes and in a unique experiment.

The amount of N-absorbed mainly depends on SNU and root system (Garnett et al., 2009; Ulas et al., 2012; He et al., 2017). Although genotypes varied significantly in the amount of N absorbed, we did not find any significant differences in SNU. Moreover, although the fine root ratio remained constant during the vegetative growth regardless of N availability, this trait exhibited large genotypic differences (FineR/RDM; Figure IV.1 equation 9). Therefore, we confirmed that winter oilseed rape genotypic differences on NUpE were mainly explained by fine root ratio (FineR/RDM) rather than by differences in SNU.

The efficiency of N conversion into plant area (**PA/QNS; Figure IV.1 equation 4**) remained constant during the vegetative growth, and did not significantly vary between N-conditions. Indeed, 96% of the plant area variations up to flowering were correlated to whole plant N-quantity, whatever N-condition. Besides, this parameter exhibited a variation of 30% between genotypes (Figure IV.7). This result was in line with the results found in *Arabidopsis thaliana* (Richard-Molard et al., 2009). However, there is still some debate as to whether biomass accumulation or leaf area expansion determines nitrogen accumulation, as discussed in oilseed rape by Lemaire et al. (2007).

4.4. Towards a whole plant model for oilseed rape valid for the whole growth cycle

Vegetative growth is an important phase determining yield potential elaboration in winter oilseed rape (Diepenbrock, 2000; Bouchet al. 2016). Accordingly, biomass production and N-uptake before flowering appear crucial for final yield (Yau and Thurling, 1987; Cramer, 1993; Colnenne et al., 2002; Svecnjak and Rengel, 2006a). Nitrogen supply from vegetative tissues is the main N source to match N requirements during pod filling (Malagoli et al., 2005). We highlighted that a model-based phenotyping approach has proven to effectively hierarchize the main processes and traits underlying genotypic differences in biomass accumulation during the vegetative growth. Biomass accumulation during the vegetative growth exerts an early contribution on final N-use efficiency, and NUE-components (N-uptake and N-utilization efficiencies). However, it would be necessary to implement the proposed conceptual framework in a computer model. An operating model would extend this study to a wider range of conditions and assess the importance of parameters through sensitivity analysis. A whole-plant model simulating biomass accumulation up to the end of vegetative growth might help ranking the genotypic performances based on NUE_DM. Indeed, NUE_DM at the end of the vegetative growth (BBCH 59) was correlated to final NUE.

Extending the proposed modeling framework, up to seed maturity, might help to decipher reproductive processes involved in NUE genotypic variability. Indeed, Allirand et al. (2011) highlighted the importance of the dynamics of lateral branching, branch characteristics, pod setting, and seed growth in deciphering the processes involved in genotype x nitrogen interactions from flowering to maturity. Accordingly, our results confirm and quantify the importance of stem and pods contributions to whole plant biomass, especially as radiation interception surfaces. However, in our study we did not measure branches area. Accounting for branches area (*i.e.* projected area) and including additional relationships *i.e.*, supporting seed set or abortion and seed filling, might be the first step to extend the model relationships at least during pod development and ideally until harvest. This will also help to decipher NUE elaboration during the reproductive phase.

SUPPLEMENTARY DATA

Table IV.S1. Effect of the N condition on the measured plant state variables used in the conceptual modeling framework over the plant growth cycle for (A) biomass and plant area, and (B) carbon and nitrogen related variables. The following growing stages were targeted: from rosette growth until beginning of the main stem elongation (BBCH 19 to BBCH 32), from the stem elongation (BBCH 32) just before flowering (BBCH 59), from the end of flowering through pod development (BBCH 68-71) and seed maturity (BBCH 88-89). At each sampling stage, mean values of the state variables were expressed by averaging the data (n= 6 to 7) of AVISO plants cultivated in the same experiment (GR15 and GR18) and N-condition. The significance of the nitrogen condition (N) and (G) effect was assessed for each experiment separately. Significant codes: *** P-value<0.001, ** 0.01<P-value<0.001, * 0.05<P-value. NS non-significant. NA: not available.

A.

Variable Stage	Units	GR15				GR18			
		N0	N1	N2	N	N0	N2	N	G
Plant dry matter ($g\ plant^{-1}$)									
BBCH 16	na	na	na			na	na		
BBCH 19	1.50	1.71	2.33	*	0.36	0.35	ns	***	
BBCH 20	5.99	6.30	7.16	ns	3.42	3.87	ns	***	
BBCH 21	9.42	10.48	10.30	ns	na	na			
BBCH 30-32	10.57	11.46	17.84	***	na	na			
BBCH 59	14.07	18.86	28.12	***	8.52	13.45	***	ns	
BBCH 68-71	29.59	35.41	41.50	***	16.27	41.42	***	ns	
BBCH 88-89	36.79	41.45	50.71	*	na	na			
					34.05	82.09	***	*	
Shoot dry matter ($g\ plant^{-1}$)									
BBCH 16	na	na	na		0.26	0.27	ns	***	
BBCH 19	1.13	1.31	1.88	**	2.46	2.87	ns	***	
BBCH 20	4.61	4.65	5.88	ns	na	na			
BBCH 21	6.62	7.92	8.38	ns	na	na			
BBCH 30-32	7.14	8.11	13.30	***	6.08	10.79	***	ns	
BBCH 59	8.86	12.48	21.09	***	12.23	33.63	***	ns	
BBCH 68-71	23.09	27.77	33.52	***	na	na			
BBCH 88-89	30.67	35.07	41.76	**	30.75	74.87	***	*	
Root dry matter ($g\ plant^{-1}$)									
BBCH 16	na	na	na		0.10	0.08	*	***	
BBCH 19	0.37	0.40	0.45	ns	0.96	1.00	ns	***	
BBCH 20	1.38	1.65	1.28	ns	na	na			
BBCH 21	2.96	2.56	1.91	ns	na	na			
BBCH 30-32	3.44	3.35	4.54	*	2.44	2.67	ns	ns	
BBCH 59	5.21	6.39	7.04	*	4.04	7.79	***	ns	
BBCH 68-71	6.49	7.65	9.25	**	na	na			
BBCH 88-89	5.78	6.39	8.95	*	3.29	7.22	***	**	
FineRDMcum (g)									
BBCH 16	na	na	na		6.91	7.19	na	na	
BBCH 19	50.58	37.22	56.24	na	na	na			
BBCH 20	156.01	165.55	168.29	na	na	na			
BBCH 21	371.82	394.78	347.66	na	90.98	93.90	na	na	
BBCH 30-32	575.98	651.47	566.40	na	442.46	459.20	na	na	
BBCH 59	801.27	998.18	922.34	na	858.29	1240.48	na	na	
BBCH 68-71	na	na	na		na	na			
BBCH 88-89	na	na	na		na	na			
Plant Area (cm^2)									
BBCH 16	na	na	na		5184.2	4114.0	na	na	
BBCH 19	35942.4	47180.6	76742.9	na					
BBCH 20	140636.6	152511.8	239351.8	na					
BBCH 21	283635.5	306887.2	408945.7	na	59811.7	68538.4	na	na	
BBCH 30-32	329241.4	345047.4	557697.6	na	171384.1	210867.7	na	na	
BBCH 59	395954.6	478880.5	748289.3	na	285004.0	566181.8	na	na	
BBCH 68-71	589367.1	774302.2	1268976.0	na					
BBCH 88-89	651372.0	854626.9	1473293.0	na	471385.2	868360.1	na	na	

B.

Variable Stage	Units	GR15				GR18			
		NO	N1	N2	N	NO	N2	N	G
Plant N-quantity (<i>g plant⁻¹</i>)									
BBCH 16	na	na	na			0.02	0.02	ns	***
BBCH 19	0.07	0.08	0.12	**		0.11	0.14	ns	***
BBCH 20	0.18	0.19	0.28	*		na	na		
BBCH 21	0.23	0.29	0.34	*		na	na		
BBCH 30-32	0.24	0.32	0.50	***		0.25	0.44	***	ns
BBCH 59	0.30	0.38	0.71	***		0.30	0.90	***	ns
BBCH 68-71	0.35	0.46	0.92	***		na	na		
BBCH 88-89	0.38	0.52	0.92	***		0.38	1.15	***	ns
Shoot N-quantity (<i>g plant⁻¹</i>)									
BBCH 16	na	na	na			0.02	0.02	ns	***
BBCH 19	0.06	0.07	0.11	**		0.09	0.12	**	***
BBCH 20	0.15	0.15	0.24	**		na	na		
BBCH 21	0.16	0.23	0.28	**		na	na		
BBCH 30-32	0.17	0.22	0.38	***		0.18	0.36	***	ns
BBCH 59	0.22	0.28	0.56	***		0.24	0.78	***	ns
BBCH 68-71	0.29	0.38	0.79	***		na	na		
BBCH 88-89	0.32	0.45	0.81	***		0.35	0.79	***	ns
Root N-quantity (<i>g plant⁻¹</i>)									
BBCH 16	na	na	na			0.00	0.00	*	***
BBCH 19	0.01	0.01	0.01	ns		0.03	0.03	ns	***
BBCH 20	0.04	0.04	0.04	ns		na	na		
BBCH 21	0.08	0.07	0.06	ns		na	na		
BBCH 30-32	0.07	0.09	0.12	*		0.07	0.09	*	*
BBCH 59	0.09	0.10	0.12	*		0.06	0.13	***	**
BBCH 68-71	0.06	0.08	0.14	***		na	na		
BBCH 88-89	0.05	0.07	0.12	***		0.03	0.36	***	*
Plant C-quantity (<i>g plant⁻¹</i>)									
BBCH 16	na	na	na			0.18	0.16	ns	***
BBCH 19	0.55	0.62	0.82	ns		1.57	1.81	ns	***
BBCH 20	2.20	2.20	2.56	ns		na	na		
BBCH 21	3.79	4.14	4.03	*		na	na		
BBCH 30-32	4.26	4.57	6.98	**		4.21	6.39	***	ns
BBCH 59	5.67	7.53	10.96	***		7.61	18.53	***	ns
BBCH 68-71	12.59	14.80	17.48	***		na	na		
BBCH 88-89	17.37	19.51	23.39	**		16.72	38.23	***	ns
Shoot C-quantity (<i>g plant⁻¹</i>)									
BBCH 16	na	na	na			0.14	0.14	ns	***
BBCH 19	0.41	0.48	0.67	**		1.29	1.56	ns	***
BBCH 20	1.67	1.70	2.09	ns		na	na		
BBCH 21	2.60	3.11	3.29	ns		na	na		
BBCH 30-32	2.90	3.26	5.31	***		3.41	5.54	***	ns
BBCH 59	3.59	5.03	8.36	***		6.25	16.05	***	ns
BBCH 68-71	9.83	11.68	13.94	***		na	na		
BBCH 88-89	14.73	16.83	19.71	**		15.30	35.15	***	ns
Root C-quantity (<i>g plant⁻¹</i>)									
BBCH 16	na	na	na			0.03	0.03	*	***
BBCH 19	0.14	0.14	0.15	ns		0.29	0.25	*	***
BBCH 20	0.55	0.69	0.52	ns		na	na		
BBCH 21	1.13	1.02	0.74	ns		na	na		
BBCH 30-32	1.37	1.31	1.67	ns		0.80	0.85	ns	ns
BBCH 59	2.08	2.50	2.60	ns		1.35	2.48	***	ns
BBCH 68-71	2.76	3.12	3.53	*		na	na		
BBCH 88-89	2.49	2.68	3.67	*		1.42	3.09	***	**

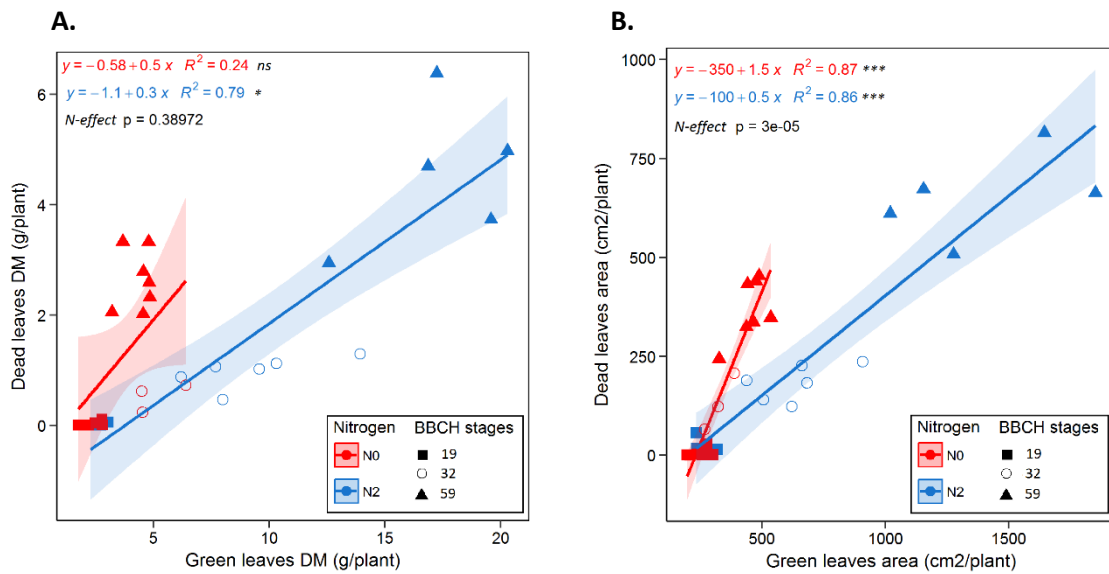
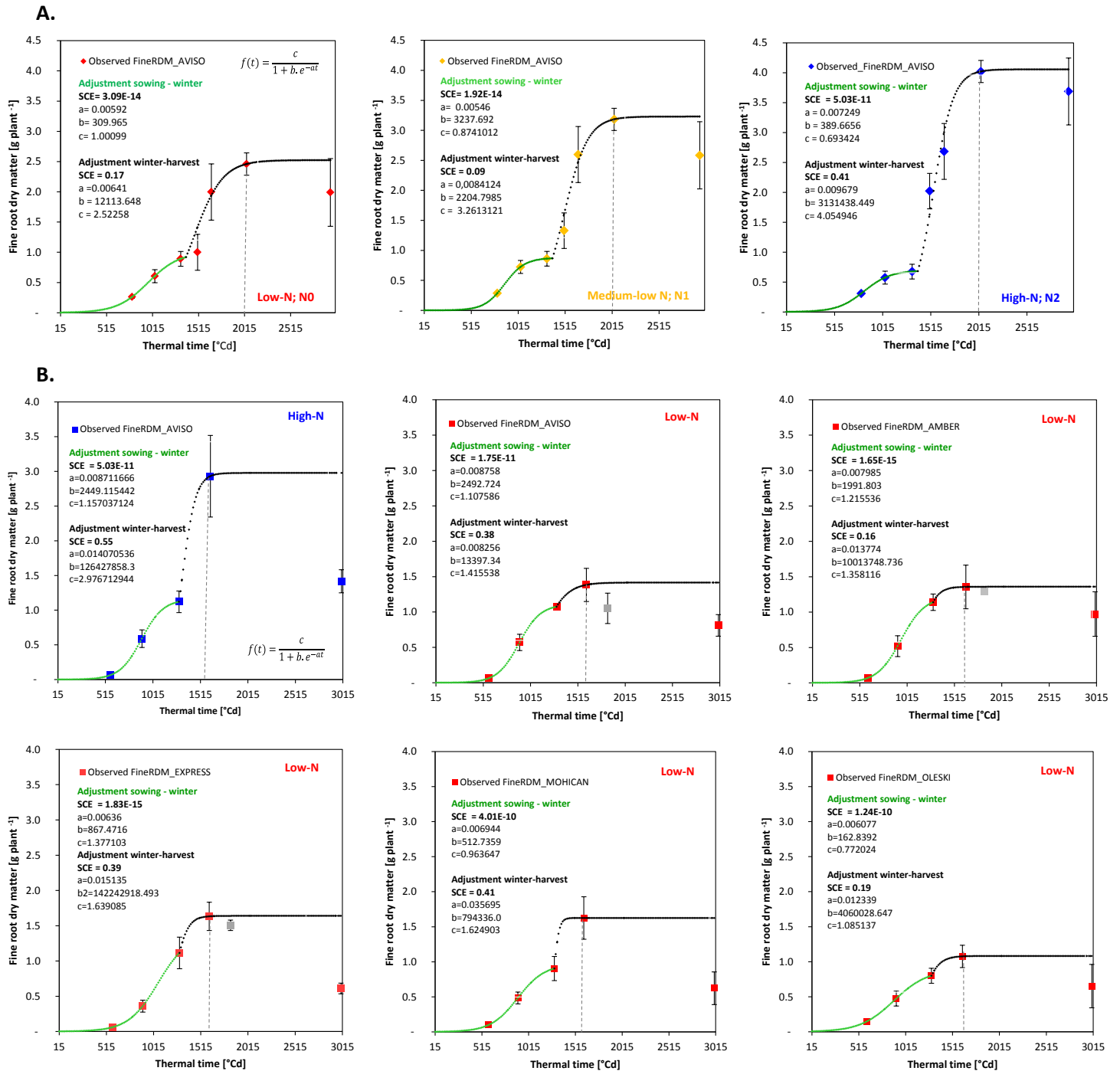
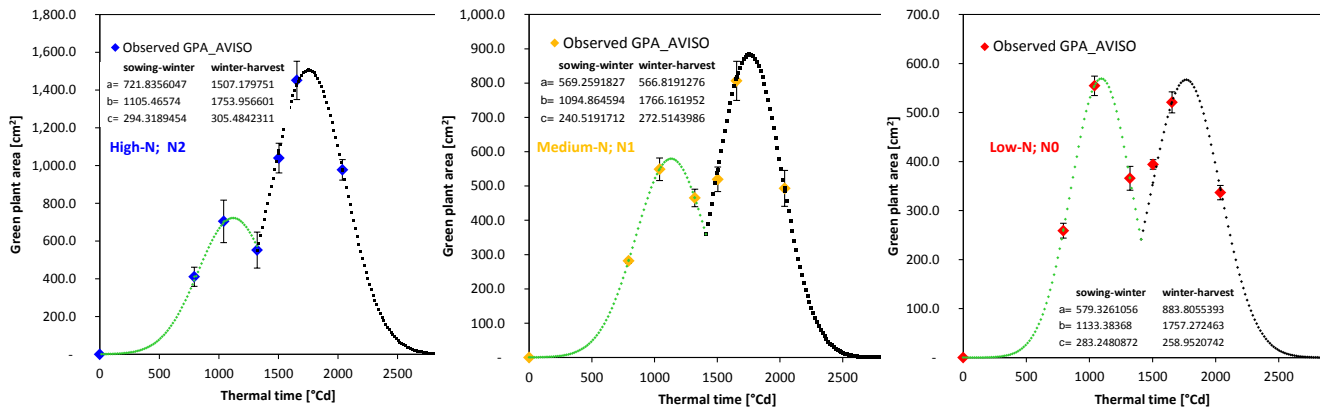


Figure IV.S2. Dry matter (A) and area (B) of the fallen leaves, as a function of dry matter and area of green leaves respectively, for Aviso grown under low-N (N0) and high-N (N2) conditions in the GR18 experiment. Colors correspond to N-conditions (red, low-N; blue high-N) and colored shaded areas indicate the 95% confidence interval of the linear regression. Symbols correspond to the growing stages of each single plant measurement. The regression equations and coefficients of determination (R^2) are indicated on the figures. The number of stars indicates the significance level of the linear regression. P-values correspond to the N-effect on the parameters. Significant codes: *** p value<0.001, ** 0.01<p value<0.001, * 0.05<p value. NS non-significant.



IV.S3 Dynamics of fine roots during the whole plant growth cycle for A) AVISO growing under three contrasted N-conditions (limiting N0, red; medium-N N1, yellow; non-limiting N2, blue) in the GR15 experiment, and B) for the cv AMBER, EXPRESS, MOHICAN and OLESKI growing under limiting N-conditions and cv AVISO growing under limiting and non-limiting N-conditions in the GR18 experiment. Vertical grey dashed lines depict the limit of validity of the adjusted logistic equations. Error bars indicate the standard error of the mean.

A.



B.

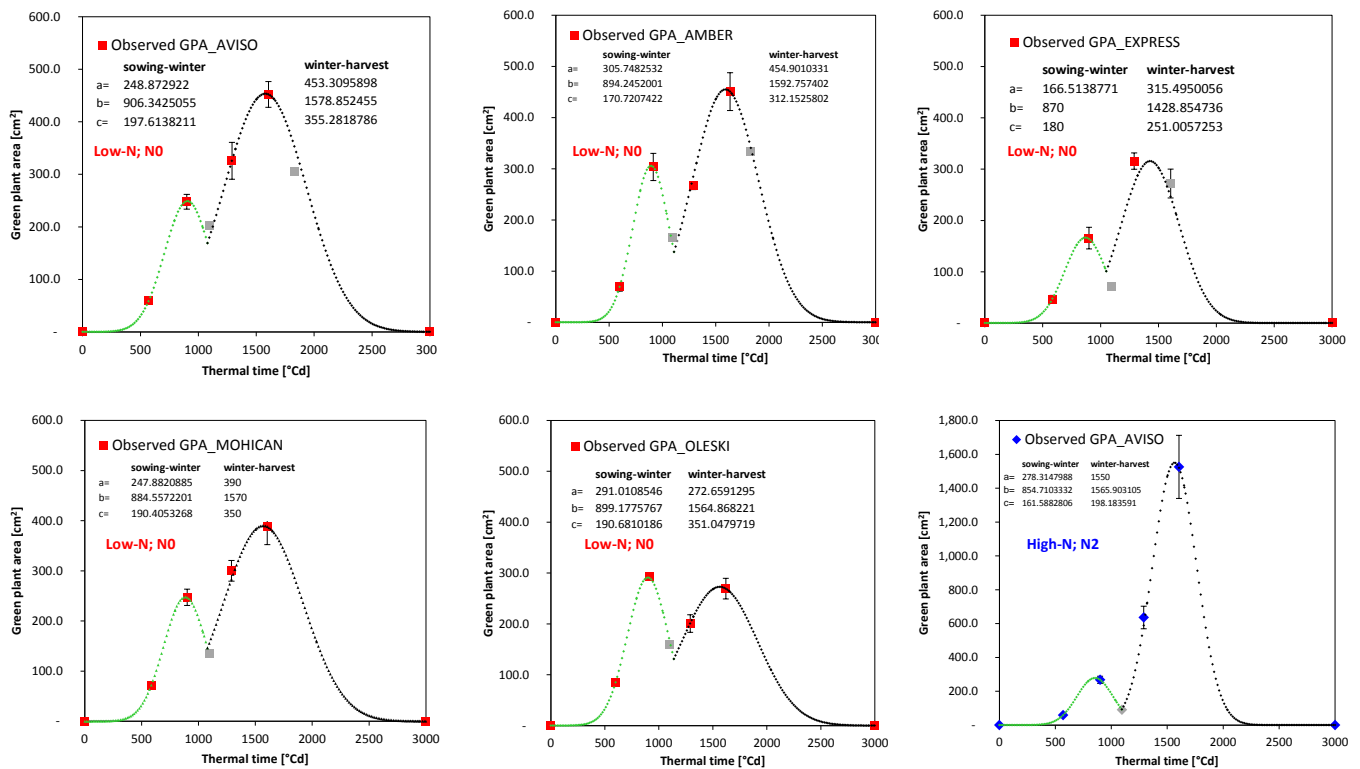


Figure IV.S4 Dynamics of plant green area during the whole crop cycle for A) AVISO growing under three contrasted N-conditions (Low-N N0, red; medium-N N1, yellow; High-N N2, blue) in the GR15 experiment, and for B) AMBER, EXPRESS, MOHICAN and OLESKI growing under limiting N-conditions and AVISO growing under limiting and non-limiting N-conditions in the GR18 experiment. Green curves correspond autumnal growth and overwintering period, whereas black curves correspond to growth recovering after winter up to seed maturity. Error bars indicate the standard error of the mean. Green plant area includes surfaces of green leaves, main stem and pods, when present. Branches area was not measured.

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Overview and highlights of Chapter IV.

In this chapter, we proposed a first whole-plant conceptual modeling framework for winter oilseed rape to explain C and N fluxes between plant organs during the vegetative period. We used it i) to assess whole-plant processes supporting the observed genotypic variation in response to N-conditions, and ii) to reduce and hierarchize the source of genotypic variation of plant responses under the low-N condition to a limited number of traits underlying biomass accumulation. Considering that NUE might be expressed as the ratio of produced biomass to N-available, a model that report the dynamics of whole plant biomass accumulation during the growth cycle might be used to calculate NUE.

This simple conceptual modeling framework has proved to be a relevant tool for dissecting the stream of physiological and environmental events leading to contrasting plant behaviors in response to N-conditions. Assessing genotypic variation under low-N conditions through model parameters seems to be more relevant than studying dynamic state variables, measured punctually over the plant growth cycle, such as biomass, surfaces, or specific C and N contents.

Ours results pointed out that winter oilseed rape response to N-conditions was mainly supported by carbon assimilation efficiency (SCA), specific N-uptake (SNU) and part of fallen leaves to plant area (FLA/PA). In contrast, genotypic variation under low-N conditions was supported by SCA, carbon partitioning between leaves and stems, and fine root ratio (FineR/RDM). As no difference was observed for SNU, we suggested the fine root ratio as the main parameter underlying the genotypic variation in N-uptake efficiency.

The next step will consist of implementing the proposed conceptual modelling framework and assess the sensitivity of plant biomass and its components to the targeted parameters. The model might then be used for high-throughput phenotyping at early stages and to further evaluate the impact of roots growth on early NUE-related processes.

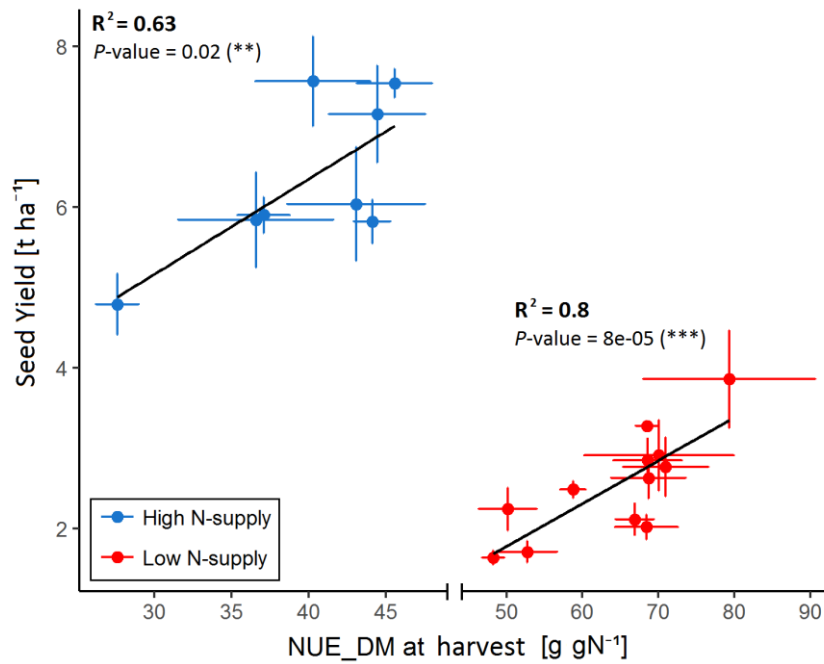


Figure V.1. Seed yield as a function of NUE_DM at harvest (BBCH 84-89) at low-N (red) and high-N (blue) conditions. Regression analyses were performed gathering data of the three experimental sites and years (LR15, GR15 and GR18). Coefficients of determination (R^2) and significance of the regression are indicated on the figures. Error bars indicate standard error of the mean.

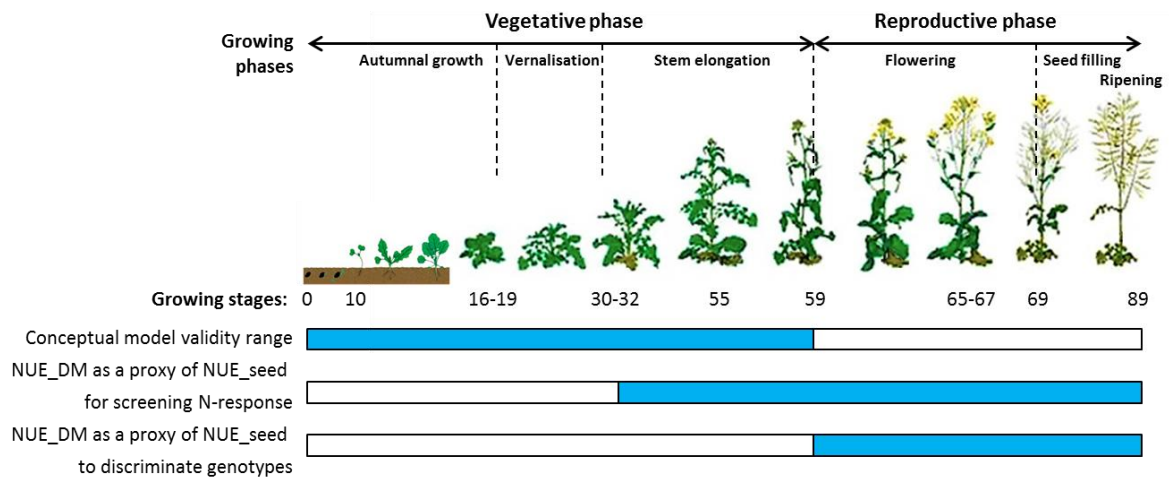


Figure V.2. Complementarity of the results from chapter III (NUE_DM at BBCH 32 or 59 as a proxy trait of NUE at harvest) and chapter IV (conceptual framework explaining oilseed rape functioning during the vegetative growth).

CHAPTER V. GENERAL DISCUSSION AND PROSPECTS

In this chapter, we pointed out the main outputs of this Ph.D. study and discussed them by combining the outcomes of Chapter III and Chapter IV, as well as the reviewed knowledge from Chapter II. The limits of this study are presented along with the new challenges the thesis raises.

5.1. Whole-plant dry matter NUE: a key variable for deciphering the dynamics of genotype response to nitrogen availability

Nitrogen use efficiency has generally been assessed using integrative indicators such as NUE_Seed (seed yield per unit of N supply) and N-harvest index (ratio of seed N quantity to above-ground N quantity) (Berry et al., 2010; Schulte auf'm Erley et al., 2011; Nyikako et al., 2014). However, these indicators can only be calculated at the end of the crop cycle. Their integrative nature makes them sensitive to non-N-related processes occurring during the crop cycle, such as plant growth, abiotic stresses (water deficit or lodging, high temperature, etc.), and biotic pressures (Bouchet et al., 2016; Corlouer et al., 2019), and complicates the estimation of the impact of some underlying physiological processes involved in genotype response to N availability and biomass elaboration.

In this Ph.D. study, we strived to describe the dynamics of N-use efficiency in response to N-availability throughout the plant growth cycle to identify physiological processes driving final differences in seed yield and NUE observed at harvest. Indeed, we approached NUE by studying the dynamic of biomass elaboration in response to N-availability. Given this goal, we proposed a novel NUE indicator, called dry matter NUE (NUE_DM), with the advantage of being quantifiable at any phenological stage and of considering whole-plant dry matter variation (including fallen leaves and fine roots). Such a comprehensive analysis is still lacking in the literature, in particular because fine roots are usually neglected on plants older than seedlings. NUE_DM at harvest was positively correlated with seed yield (Figure V.1). This relationship was obtained by gathering data between sites, years, and genotypes and was N-dependent. Indeed, NUE_DM at harvest explained 80% of seed yield variability under low N availability ($R^2 = 0.8$, $P\text{-value} = 8 \cdot 10^{-5}$), highlighting the robustness of this indicator for breeding purpose of improving seed yield, especially under low-N conditions.

The first output of this Ph.D. study is the identification of early NUE_DM as a relevant proxy trait for NUE_Seed at harvest, able to discriminate genotypes from BBCH 59 (immediately before flowering) and N-response from BBCH 32 (beginning of the stem elongation). This result highlighted the early determination of NUE and pointed to the role the vegetative phase in subsequent establishment of final NUE. This proxy trait was valid for all the climatic conditions observed through our three experiments (Chapter III, Figure 2), highlighting the robustness of this indicator for breeding purpose of improving seed yield as well as NUE_Seed. However, during autumn growth (BBCH 16-19), no correlation was found between NUE_DM and NUE_Seed at harvest. This might be explained by the fact that some genotypes (e.g. OLESKI) exhibited high growth rates during the first weeks of growth, which may have been quickly limited by soil N availability.

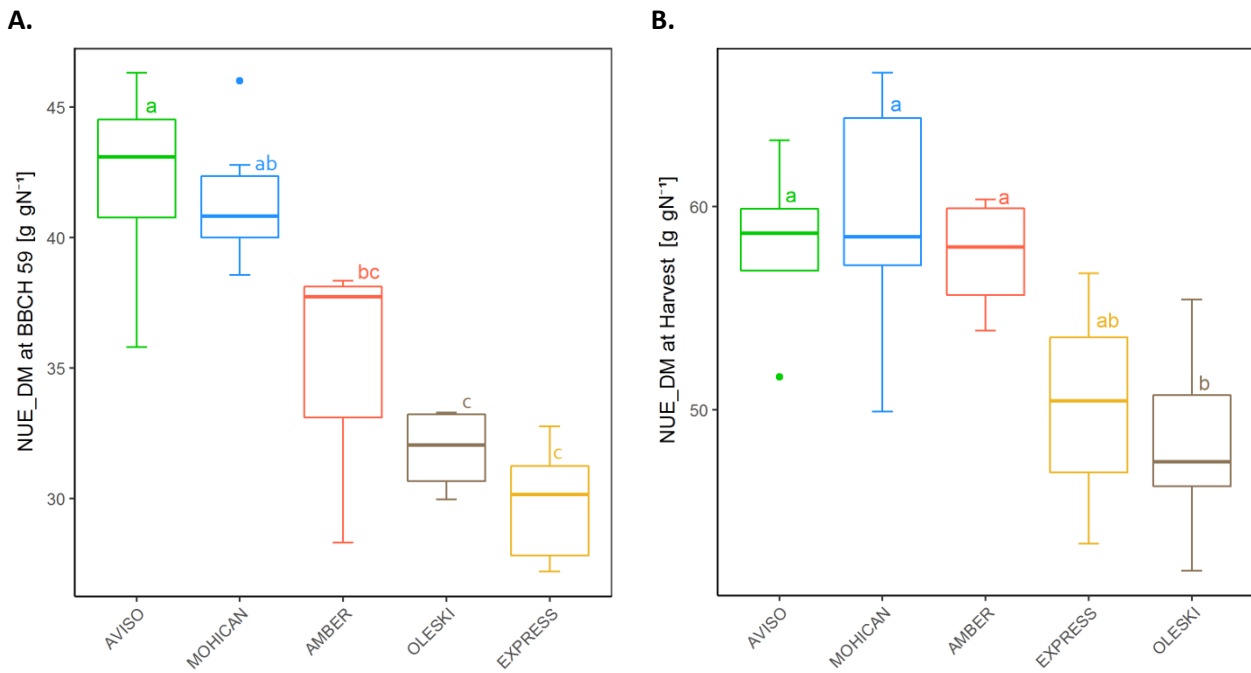


Figure V.3. Ranking of five winter oilseed rape genotypes grown under low N-conditions for N-use efficiency (NUE_DM) (A) just before flowering (BBCH 59) and (B) at harvest in the GR18 experiment. Colors correspond to genotypes. Boxplot followed by the same letter are not significantly different according to Tukey's test ($P \leq 0.05$).

The exclusion of earlier stages, such as BBCH16-19 in the proposed experimental approach may be a disadvantage for early phenotyping, as high-throughput phenotyping platforms often limit investigations of oilseed rape plants to the beginning of plant development (Jeudy et al., 2016). The identified correlation between NUE_Seed and early NUE_DM (BBCH 32 or 59 depending on the target), and conceptual model validity to characterize plant functioning from sowing up to BBCH 59 (Figure V.2) might be a promising tool to develop a model assisted phenotyping approach. This overlapping of the different NUE and NUE-related processes characterization methods allows characterizing NUE and its related processes along the whole crop cycle.

Moreover, we observed that the ranking of the studied genotypes by NUE was consistent between BBCH 59 and harvest but inconsistent at early growing stages (Figure V.3). This finding was in contrast to the results presented by Balint and Rengel (2008), who showed that there was little consistency between the NUE ranking of 12 spring oilseed rape varieties in the vegetative and reproductive stages, thus suggesting that screening oilseed rape genotypes for N efficiency for breeding purposes would require an assessment at maturity. However, the authors conducted their experiments using small pots (filled with 1 kg of dry soil). Therefore, the potential growth of plants might have been limited. Nevertheless, we suggested that the end of vegetative growth is an appropriate time to screen genotypes with improved NUE and seed yield at harvest. However, our results regarding genotype ranking should be validated for a larger set of genotypes and various N conditions. We highlight that even if the NUE_DM proxy was not valid as soon as BBCH 16-19, genotypes with improved NUE at early stages might exhibit a higher potential N pool in the plant, which might be potentially remobilized at later stages of growth at least to ensure the setting and growth of reproductive organs.

The conducted analysis on NUE components (NUpE and NUtE) underlying the dynamics of biomass accumulation during the whole growing cycle allowed us to identify a switch in the plant functioning just before flowering (BBCH 59). This has been attested both by decomposing the relative contribution of NUpE and NUtE to the NUE_DM (Chapter III, Figure 3) and by a model-assisted phenotyping approach (Chapter IV, Figure 1). Indeed, by using the conceptual model proposed in Chapter IV, we determined that relationships linked to C and N acquisition and partitioning were not conserved after BBCH 59, regardless of N-conditions (Chapter IV, Figure 5), confirming the BBCH 59 stage as a good time point candidate for the switch.

5.2. N uptake efficiency: the neglected side of N use-efficiency worth addressing in depth

To date, N uptake efficiency has been underestimated for improving winter oilseed rape NUE because of the assumption that N-utilization efficiency-related processes are the main drivers of NUE improvement (Rossato et al., 2001; Avice and Etienne, 2014; He et al., 2017). However, Stahl et al. (2015) did not find any genetic correlation between NUpE and NUtE either under low- or high-N conditions, arguing that both NUE components are independent and suggesting that some plant traits can be specifically related to NUpE and others to NUtE. There is therefore a need to select for genotypes that more efficiently uptake N as well as able to remobilize larger amounts of N to seeds (Górny et al. 2011).

Under low-N conditions, Berry et al. (2010) showed that, at harvest, genetic differences in NUpE underpin variation in NUE, suggesting that NUpE may be a valuable target for the creation of N-efficient genotypes. This Ph.D. study framed N-uptake-related processes as key factors in understanding genotypic variation in NUE under low-N condition. Our results show that, regardless of the N condition, NUpE played the major role in determining NUE variations during the autumn growth, a period when 60 to 80% of the plant N was absorbed. This highlights for the first time the key role of N uptake processes in the early determination of NUE. After flowering, the contribution of NUpE was still very high under the low-N conditions, which was consistent with the 4 to 30% N still taken up after flowering. NUtE played a role mainly during the reproductive phase under the high-N condition, where the NUpE and NUtE contribution were more balanced, underlying the importance of extended N uptake after flowering. The importance of N absorption after flowering had already been suggested by Berry et al., (2010), Schulte auf'm Erley et al., (2011), and Ulas et al. (2012).

However, Geiger (2009) showed for maize that the relative importance of NUpE and NUtE largely depends on the genetic base of genotypes (originated from tropical vs temperate regions) was related to different physiological mechanisms in response to N conditions. We can expect that exploring a wider range of genetic variability for *Brassica napus* L., including more exotic material such as semi-winter, spring types or swedes, may modulate conclusions about the importance of NUpE. However, these germplasms are not as directly usable in winter oilseed rape programs as the genetic variability explored in the frame of this PhD. According to Schulte auf'm Erley et al. (2011), Kessel et al. (2012), and Wang et al. (2020) oilseed rape hybrids show an improved N-use efficiency compared to inbred lines. Hybrids are of particular interest for growing oilseed rape under suboptimal N-conditions, as they exhibit higher yield stability under low-N conditions (Gehring et al., 2007), and increased yields under high-N conditions (Koeslin-Findeklee et al., 2014). The last authors compared the NUE of eleven inbred lines and seven hybrids over two years in field experiments and concluded about the superiority of hybrids for both NUpE and NUtE, resulting in higher overall N-efficiency.

This Ph.D. study sought to characterize NUpE processes in order to identify the most significant traits supporting NUE genotypic variability during vegetative growth. The combined use of experimental and model-assisted phenotyping approaches allowed us to highlight first that genotypes differing for NUpE did not vary in specific nitrogen uptake (SNU) but rather in fine root growth (Chapter III, Figures 4 and 5; Chapter IV, Table 4) and second, that SNU was submitted to N plasticity, in contrast to the fine root ratio (Chapter IV, Figure 6 and 7). Interestingly, this lack of genetic variability for SNU has not been reported either in wheat (Laperche et al., 2006), *Medicago truncatula* (Moreau et al., 2012b), or in *Arabidopsis thaliana* (Richard-Molard et al., 2009), although a similar modeling approach was used on these species. As a consequence, we suggest that oilseed favor plasticity of subterranean structures to adapt to environmental changes rather than contrasting functional abilities, as opposed to other species, similarly as it has been shown for the aerial structures (e.g. Pinet et al., 2015).

5.3. Lighting the dark side of winter oilseed rape: why fine roots matter in Nitrogen uptake efficiency

Most authors have underlined the crucial role of the root system in NUE and N uptake (Kamh et al., 2005; Stahl et al., 2016). However, under field or field-like conditions, root system characterization was usually limited to taproots at best (Sieling et al., 2017; Arifuzzaman et al., 2019). Our results recognize the absence of a correlation between fine roots and taproots (Chapter III, Figure 5). We therefore highlight that neither taproot biomass nor the total root biomass could be used as a proxy of fine root biomass or NUpE to screen for genetic variability, emphasizing the importance of exhaustively phenotyping the fine root system. However, genetic control of root traits remains poorly understood and highly quantitative, with important genotype and environment (G x E) interactions (Wang et al 2017; Lynch, 2019; Louviaux et al., 2020).

Schulte auf'm Erley et al. (2011) concluded in their research on NUE in winter oilseed rape that mechanisms to achieve high N uptake could be manifold. Fine-root growth depends on carbon allocation in the root compartment and hence on whole-plant functioning. Using a model-assisted phenotyping approach, we propose specific carbon assimilation as a primary shoot-related process supporting observed genotypic variation in response to N conditions. This parameter determines the whole-plant C quantity and therefore the potential pool of C that might be allocated to root growth, highlighting the crucial impact of considering both carbon — and nitrogen—allocation in at both shoot and root compartments. The requirement for whole-plant studies has already been advanced by Brun et al. (2010)—who suggested that most of the modifications of the root system architecture in response to limiting N conditions were driven by modifications in aerial C fluxes in *Arabidopsis thaliana*—and by Faverjon et al. (2018)—who highlighted that concomitant analysis of root and shoot development is necessary to properly decipher rooting strategies in forage legumes.

In this Ph.D. study we showed that fine root biomass was highly correlated to NUpE during autumn growth (Chapter III, Figure 5), explaining up to 92% of the genotypic variation observed in N uptake efficiency under the low-N condition. Fine root biomass might, therefore, be a relevant trait to characterize NUpE performance of oilseed rape genotypes. The emergence of fine roots as a promising lever of NUE genotypic improvement also raises new issues for root phenotyping methods. The research on below-ground traits is complicated but has recently been gaining interest among breeders (Lammerts van Bueren and Struik, 2017; Lynch, 2019). However, the literature on below-ground traits in oilseed rape in particular remains limited and not all available research on below-ground traits directly relates such traits to NUE (Lammerts van Bueren and Struik, 2017). Han et al. (2015) stressed the relative importance of accurate phenotyping and experimental designs over genotyping for such complex traits (Chapter II., section 2.5. Phenotyping for NUE). Moreover, while the aerial organs are considered in breeding programs, examinations of root system are receiving much less attention because of the practical challenge of working with the below-ground organs (Araus et al., 2014). Destructive phenotyping at different developmental stages through the crop cycle, as performed in this study simultaneously for shoot and root compartments, can help to identify potential traits underlying NUE genotypic variations to N conditions. However, this approach is extremely time-consuming and cost-intensive, and thus not suitable for analyzing thousands of genotypes in breeding programs (Postma et al., 2014). The development of suitable devices for the high-throughput phenotyping of root stem remains a major bottleneck (Nguyen and Kant, 2018). Thus far, most of the techniques developed for high-throughput root phenotyping involve the use of seedling plants. Although oilseed rape seedling root traits have been linked to seed yield (Koscielny and Gulden, 2012; Thomas et al., 2016), our results pointed out that it remains crucial to phenotype older stages both for assessing NUE elaboration (BBCH 32) and for estimating the genotype ranking for NUE at seed maturity (BBCH 59). Winter oilseed rape, as a dicotyledonous plant, develops a long taproot, making it impossible to grow plants to seed maturity in classic small pot experiments without limiting root growth (Hohmann et al., 2016). Indeed, adopting a standardized definition of fine roots is a critical first step in advancing fine root phenotyping, according to recent reviews by McCormack et al. (2015), Prieto et al. (2015), and Laliberté (2017). In our study, we defined fine roots using a diameter-based classification (≤ 0.2 mm in diameter), but studies defining the fine root threshold are scarce.

In the field, rhizotrons have been widely used because they allow the dynamic characterization of root growth rate. However, current tools for processing rhizotron images are often restricted to a limited number of variables and show limitations with highly branched root systems (Lobet et al. 2013). To cope with these phenotyping constraints, adapted plant phenotyping devices and platforms have been developed such as the Plant Phenotyping Platform for Plant and Micro-organism Interactions (4PMI). This platform allows shoot and root high-throughput phenotyping by combining both non-invasive and destructive methods for about 1,500 plants growing in tubes 50 cm deep (RhizoTubes; Jeudy et al., 2016) under controlled conditions. In a previous study (Cordier and Richard-Molard, 2016), oilseed rape plants were successfully cultivated in the 4PMI under contrasting N-nutrition levels up to BBCH 16-19 (488 growing degree days), where plants occupied the maximum soil volume. This experimental device allowed for the quantitative characterization of shoot and root morphology traits, but only up to early stages (BBCH 16).

In perspective, more genotypes with contrasting root morphologies could be screened to further strengthen the relationship between root architecture and NUpE. Indeed [Louvieux et al. \(2020\)](#) conducted a field study in oilseed rape and suggested that NUpE could be improved by favoring a more branched root system that explores a larger soil volume.

The oilseed rape modeling framework developed in this Ph.D. study allows to simulate fine root dry matter throughout the vegetative growth, up to BBCH 59. We hypothesize that combining both high-throughput phenotyping (HTP) experimental measurements and our modeling framework might allow for the precise characterization of fine root traits at early stages, simulate growth at later stages, and evaluate fine roots impact on NUE elaboration. Moreover, given that GxE interactions significantly interfere with HTP of below-ground traits *per se* (reviewed by [Bouchet et al., 2016](#) and [Lammerts van Bueren and Struik, 2017](#)), this model-assisted phenotyping approach could be adapted to overcome uncontrolled environmental variation ([Postma et al., 2014](#); [Gu et al., 2014](#)).

To further analyze the impact of fine roots on NUpE, a characterization of the root system architecture would be relevant ([Shi et al., 2018](#); [Pagès, 2019](#)). Root architecture is a major determinant for nutrient uptake and therefore has a considerable impact on winter oilseed rape NUE ([Wang et al., 2017](#)). Indeed, as root growth and maintenance are energetically costly, the root architecture might optimize the balance between nitrogen absorption ability and metabolic costs ([Lynch 2019](#)). Root architecture involves complex traits (*e.g.* root emission, elongation, and branching) that are difficult to measure under field-like conditions using current phenotyping devices and techniques. A modeling approach might be used to overcome these limitations, as suggested by [Pagès et al. \(2014\)](#), who designed a generic model called ArchiSimple that enabled the representation of architectural diversity in various plant species interacting with diverse environmental conditions. The parameters of this mechanistic model are traits with biological meaning, accounting for processes contributing to the developmental dynamics of the root system such as primary root emergence, root elongation, branching, and decay. [Moreau et al. \(2017\)](#) studied the parameters of ArchiSimple to analyze the interspecific diversity of root architecture within a range of weed and crop species, including oilseed rape, and the response of root architecture to soil-nitrogen availability. We therefore hypothesize that integrating a description of root architecture into the development of the oilseed rape whole-plant conceptual modeling framework might be relevant and useful to identifying root traits that support observed variation in fine roots, as well as underlying the observed genotypic variation for NUpE and its overall NUE impact. However, we were unable to accurately characterize root architecture with our data.

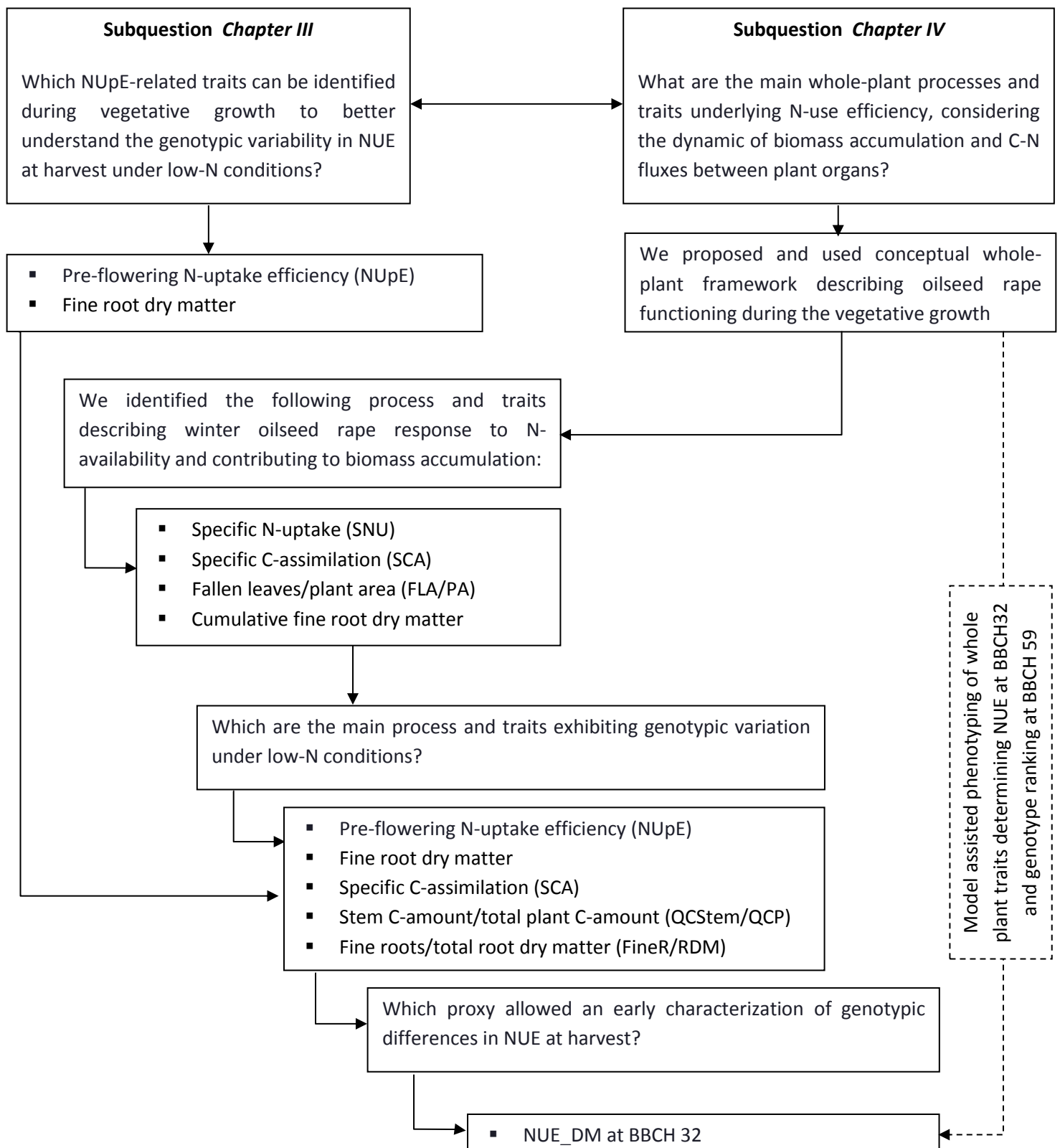


Figure V.4. Summary of the Ph.D. findings, in response to the scientific questions asked. Doted lines depict perspectives raised in the thesis regarding model-assisted phenotyping.

5.4 « Think globally, act locally » the interest of a research strategy combining targeted experimentations and whole-plant modeling framework

Whole-plant processes impacting plant C and N fluxes remain relatively unexplored in oilseed rape (Song et al., 2020). A greater research effort would foster a better understanding of processes underlying whole-plant traits, their response to the environment, and their genetic control (Lynch, 2019). Our research approach combining experiments and conceptual modeling has produced results over the whole plant cycle that are complementary in a phenotyping objective (Figure V.4). Indeed, we could estimate genotypic differences in NUE at harvest from NUE_DM measured at BBCH 59, by assimilating early phenotyping data in a future implementation of the conceptual model (Figure V.2).

Moreover, we presented and successfully used a conceptual model to associate the dynamics of NUE-related state variables over the crop cycle with efficiency and allocation parameters, which are less dependent on the environment and the compensation between processes. Assessing the variability of model parameters under contrasting N conditions (for one genotype) and the genotypic variation of parameters under low-N condition helped us identify and hierarchize the main traits determining biomass accumulation and partitioning, which drives NUE, from early stages in the growth of oilseed rape, characterized by high phenotypic plasticity. The next step might consist of characterizing and hierarchizing the model parameters determining the GxN variability of NUE. For this purpose, we suggest to conduct an experiment combining i) contrasting growing N-conditions, ii) a wide range of genotypes, iii) measurements of whole plant biomass (including fine roots and fallen leaves) and above-ground area (including main stem and fallen leaves), and iv) multiple samplings during vegetative growth for the adjustment of dynamic curves of fine root dry matter, plant green area and fallen leaves area. Indeed, we suggest conducting at least one sampling at BBCH 16-18, BBCH 30-32 and BBCH 59 (Figure IV.S3 B and S4 B), and if possible, and additional sampling at the beginning of winter for accounting for growth stagnant during overwintering period (Figure IV.S3 A and S4 A). Although the LR15 experiment met the requirements (i) and (ii), there was a lack of fallen leaves and main stem area phenotyping and of the minimum sampling frequency for dynamic growing adjustments.

Nevertheless, we noticed that the response of winter oilseed rape to N conditions was supported by carbon assimilation efficiency (SCA), relative contribution of fallen leaves to total plant area (FLA/PA), and specific N uptake (SNU). Despite SCA mainly supporting the observed genotypic variation to low-N conditions, no genotypic variation was observed for SNU, suggesting fine root ratio as an underlying N uptake efficiency genotypic variation. However, root phenotyping is a current challenge for plant scientists (Lynch, 2019), especially under field conditions and branched root systems (Lobet et al. 2013). The next step will be to progress from the conceptual framework to a computer model and analyze the sensitivity of the whole set of parameters, especially SNU and SCA, which we hypothesize might be linked to the produced biomass, as reported by Richard-Molard et al. (2009) in *Arabidopsis thaliana*.

As reviewed in Chapter II, canopy architecture involves interactions among the organs of a single plant as well as interactions among plants. Those interactions should be considered when studying the response of oilseed rape to N availability (Bouchet et al., 2016). Indeed, considering the compensatory capacities of the plant's components between the vegetative and the reproductive phase, plant-scale processes impacting carbon and nitrogen fluxes deserve to be studied under field-like conditions.

We selected the PERISCOPE phenotyping culture device (Bissuel-Belaygue et al., 2015) as it allows for growing oilseed rape plants during the whole crop cycle under field-like reconstructed canopies, obtaining phenotypes and seed yields similar to those of field-grown plants but allowing quantitative access to each plant fraction, including fine roots. However, we cannot overlook the fact that even if the PERISCOPE device considers the interaction between plants at the shoot level, there are no interactions between plants at the soil level, which makes this device ambivalent for root phenotyping. On the one hand, this device allows access to each plant's root system, enabling sampling of branched fine root systems. On the other hand, root system interactions between plants that might affect carbon and nitrogen fluxes are not considered.

In this Ph.D. study, we highlighted the importance of accurately quantifying the soil N availability when characterizing and discussing NUE (Chapter II). As nitrogen needs to be available in the space where crop roots can take it up, inaccurate estimation of soil N availability will directly impact NUE's values. Indeed, when N availability is underestimated, NUE values will be higher than expected. In our experiments, although we carefully irrigate plants using a simulated water balance to reduce drainage, and consequently N-losses, each tube evacuates potential excess nutrient solution through a hole in its base. We cannot wholly exclude potential N-losses by leaching, but no measurements were performed to quantify it accurately. However, as we supplied all the experimented plants with the same volume of mineral nutrition, our comparisons remain valid in relative terms (*i.e.* within the conducted experiments). A current methodological challenge will be to release the N lixiviate from the bottom to the top of the tube (*e.g.* Jeudy et al., 2016) or, at least, quantify the N lixiviate volume (*e.g.* Hohmann et al., 2016) and its N content with higher accuracy. In our study, we avoid water and other nutrient stress, except N. Indeed, we supplied N-nutrition solution during the whole crop growth-cycle at different quantities to generate either a constant deficit or N-surplus. The conducted irrigation and N-management strategy allowed us to isolate N from other potential stresses affecting N-availability and plant growth (*i.e.* water stress). However, under field conditions, water availability might have an impact on N-uptake. Water is considered one of the most critical factors influencing soil nutrient availability and microbial activity, which contribute to N-mineralization and N-availability for the crop. Besides, water is the medium of nutrient transport in soil, so although nutrient and water uptake are independent processes, they are inextricably linked (Guntinas et al. 2013). The next step might be the validation of our results under field conditions. However, as fine roots phenotyping remains a challenge under field conditions, a first step might be to consider studying the identified processes and traits combining N and water regimes under semi-controlled conditions. Besides, the complexity of improving NUE requires the integration at the canopy level and calls for more research on other factors influencing the uptake process such as root exudates, available rhizobium, or nitrate transport systems (Lammerts van Bueren and Struik, 2017).

5.5. Toward growing oilseed rape for sustainable agriculture production

Altogether, the results of this Ph.D. study suggest considerable scope for (i) improving N use efficiency in oilseed rape under low-N conditions by targeting N uptake efficiency and fine root ratio and (ii) for using a model-assisted phenotyping approach to assess the impact of the candidate traits on overall plant functioning.

In our study, we evidenced significant differences between genotypes AVISO and EXPRESS considering fine root dry matter and fine root contribution to the whole root system. Thus, the mapping population of 200 inbred lines obtained from the cross between these two genotypes seems promising to study in more details the genetic determinants of root architecture and their relation to NUE and NUpE. This population was created and genotyped in the framework of the ANR project Brassinam (ANR-10-GENM-001). It would be relevant to screen this progeny using an adapted device such as the 4PMI platform to identify the main QTL involved in NUE, NUpE and root architecture control. The expected results will help targeting candidate genes and function underlying these “meta-processes” and might also present direct interest for breeding. Indeed, diagnostic genetic markers related to root traits QTL would allow to screen germplasm without having to go through a deep phenotyping process.

From a breeding point of view, phenotypes with improved root systems are promising breeding targets. Root traits that reduce the metabolic cost of soil exploration (*i.e.* root hairs) should be prioritized in breeding programs seeking to develop N-efficient crops urgently needed in global agriculture (Lynch et al. 2019). Moreover, another complexity of breeding for NUE deals with the selection of a clear definition of the target environment. Selection for crop efficient in N capture under high-input agroecosystems is an example of an apparently fairly simple selection regime—since one resource is targeted—but one that is in fact more complex due to the multiple roles of roots (water uptake, plant anchorage, etc.). Additionally, breeding for low-input agroecosystems is more complex, because multiple stresses are often important in such systems (Lynch, 2018). Indeed, the fact that most breeding programs are conducted under optimal N conditions has resulted in genotypes that do not always perform well under low N input. The selection of these genotypes may have favored high-yielding genotypes with increased NUtE but not those with a high NUpE (Lammerts van Bueren and Struik, 2017). We therefore positioned our study in this context of selection directly under low-N conditions.

Ravier et al. (2017) reported that periods of N deficiency in wheat during vegetative growth did not lead to a decrease in yield or grain protein content and even resulted in some periods in which the N deficiency improved NUE. However, previous studies have shown that N deficiency leads to lower rates of shoot mass accumulation due to lower levels of leaf expansion (Lemaire and Gastal, 2009). Indeed, Ravier et al. (2017) concluded that the intensity of N deficiency that can be tolerated may depend on the biomass and leaf area index (LAI) of the crop at the time of the N deficiency, but they were unable to demonstrate such an effect with their data. Nevertheless, Lammerts van Bueren and Struik (2017) highlighted that knowledge and practices gained from improving NUE in one crop species cannot easily be transferred to another crop species. Short-cycle crops respond differently from long-cycle crops, and vegetative crops differ in their N management from grain-producing crops. Indeed, oilseed rape shows a large plasticity, and under constraining growing conditions, there are invariably multiple regulatory mechanisms to maintain their growth. For example, Cong et al. (2020) recently reported that applying N fertilizer after winter haze can partly compensate for the negative influence of low-radiation during vegetative growth, which leads to a decrease in photosynthetic productivity, and ensure oilseed rape yield. Indeed, another potential lever to improve winter oilseed rape NUE might consist of modifying some N-management strategies, including optimized N fertilizations adapted to dynamic crop N requirements. Although we did not focus this Ph.D. study on it, our results regarding the dynamic contributions of NUpE and NUtE to variations in NUE might help at understanding in which growing stages N might be potentially more absorbed. Indeed, elucidating the mechanisms involved in the interactions between the rhizosphere and N fertilization for oilseed rape would allow for the screening of genotypes that optimize the rhizosphere and N absorption, which could improve nutrient use efficiency in a climate and agronomic context of combined environmental stresses. Here too, a modeling approach would be useful to better understand the impact of these new practices on the response of oilseed rape to low-N conditions.

The prospect of using oilseed rape for biofuels is only economically and energetically feasible if the crop does not require intensive fertilization. To increase plant productivity under N-limited and fluctuating environmental conditions, alternative N management (*i.e.* foliar fertilization, Pużyńska et al., 2018; Rossmann et al., 2019) and agronomic practices (*i.e.* intercropping, Lorin et al., 2016) are also being developed. These new practices might impact carbon and nitrogen fluxes, thereby affecting plant functioning and NUE. For example, the association of oilseed rape with other crops (*i.e.* legume) might have several advantages regarding weed and insect impact and N supply in comparison to pure oilseed canopies. However, above-ground competition for light between crops might impact carbon production and allocation, whereas below-ground interactions might impact the deployment of root architecture and, as such, the capture of nitrogen resources, impacting overall C-N fluxes. Future evolutions of oilseed rape production will make more complex the nature and the dynamics of C-N exchanges between soil, plants, and air, as well as between plant organs. Thus, the optimization of the nitrogen use of these future cropping systems, important from an economic and environmental point of view, would necessitate combining an in-depth understanding of such complex systems with an efficient phenotyping approach to take profit of both the genetic variability and phenotypic plasticity, as partly shown by this doctoral work on a much narrower scope.

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Dissemination of Findings

SUBMITTED PUBLICATIONS

Vazquez-Carrasquer V, Bissuel-Bélaygue C, Laperche A, Chelle M, Richard-Molard C. 2020. **Nitrogen Uptake Efficiency, mediated by fine root growth, early determines variations in Nitrogen Use Efficiency of rapeseed.** Journal of Experimental Botany (submitted, under review).

CONFERENCE PRESENTATIONS

Vazquez-Carrasquer V, Bissuel-Bélaygue C, Laperche A, Chelle M, Richard-Molard C. 2019. **Deciphering the response of winter oilseed rape to nitrogen inputs: fine roots do matter in Nitrogen Use Efficiency!** 15th International Rapeseed Congress International. Oral communication, Berlin, Germany.

Vazquez-Carrasquer V, Bissuel-Bélaygue C, Laperche A, Chelle M, Richard-Molard C. 2018. **Why does it matter to consider processes instead of variables for deciphering NUE in oilseed rape? The case study of leaf area and whole-plant N quantity.** Rapsodyn annual meeting, Mondonville, France.

OTHER PUBLICATIONS OF THE CANDIDATE

Bohnert G, Vazquez-Carrasquer V, Richard-Molard C. 2018. **Phénotypage par analyse d'image de la dynamique des surfaces aériennes chez le colza : effet de la variabilité génétique et de la dose d'azote.** MSc Internship report, DARS. Montpellier SupAgro, p.46.

Chenu K, Fletcher A, Vazquez-Carrasquer V, Christopher JT. 2017. **Historical improvement in drought adaptation of Australian wheat varieties has coincided with changes in key physiological traits.** 2nd Agriculture and Climate Change Conference 2017, Sitges, Spain.

Vazquez-Carrasquer V, Christopher JT, Chenu K. 2017. **Phenotyping wheat seedling traits in contrasting temperature environments.** 2nd Agriculture and Climate Change Conference 2017, Sitges, Spain.

Vazquez-Carrasquer V, Christopher JT, Chenu K. 2016. **Effects of temperature on seedling root traits – Testing a new high-throughput phenotyping system in wheat.** ComBio2016 Conference, Brisbane, Australia.

Chenu K, Fletcher A, Vazquez-Carrasquer V, Christopher JT. 2016. **Historical improvement in drought adaptation of Australian wheat varieties has coincided with changes in biomass partitioning, transpiration efficiency and root architecture.** ComBio2016 Conference, Brisbane, Australia.

Vazquez-Carrasquer V, Christopher JT, Chenu K. 2016. **Impact of soil temperature on seedling roots in wheat and implications for adaptation to water-limited environments.** 7th International Crop Science Congress, Beijing, China.

Titre : Identification et variation génotypique des traits déterminant précocement l'efficacité d'utilisation de l'azote (NUE) du colza d'hiver sous faibles intrants azotés.

Mots clés : Écophysiologie, *Brassica napus* (L.), NUpE, variabilité génétique, indicateurs précoces, phénotypage assisté par modèle.

Résumé : Améliorer le rendement du colza dans un contexte de bas intrants azotés (N) est un enjeu majeur de sélection. Ceci impose une connaissance approfondie de la variabilité génétique des processus sous-tendant l'efficacité d'utilisation de l'azote (NUE, rendement en graines par unité d'azote disponible). Cette thèse vise à mieux comprendre les processus écophysiologiques contribuant à la NUE et à ses composantes sous faible nutrition azotée, en identifiant et hiérarchisant les principaux traits sous-tendant leur variabilité génotypique. Sept génotypes de colza d'hiver ont été étudiés en conditions semi-contrôlées sous des doses d'azote contrastées. Nous avons montré que la variable NUE_DM (biomasse totale produite par unité d'azote disponible) est un indicateur précoce de la NUE à la récolte, permettant de caractériser dynamiquement la NUE des différents génotypes à partir de BBCH 59, et en réponse à l'azote à partir de BBCH 30-32.

L'efficacité d'absorption d'azote (NUpE, N absorbé par unité d'azote disponible) s'est révélée être une composante majeure de la NUE sous contrainte azotée, expliquant 80 % des variations avant la floraison, et plus de 30 % après. De plus, sa variabilité génotypique dépend de la biomasse des racines fines et non de l'absorption spécifique d'azote. Grâce au développement d'un cadre conceptuel de modélisation du fonctionnement du colza décrivant les flux de carbone et d'azote dans la plante entière et valable jusqu'à floraison, nous avons fait ressortir l'assimilation spécifique de carbone, la part de carbone allouée aux tiges et la proportion de racines fines comme paramètres clés de la réponse génotypique à l'azote. Nos résultats suggèrent que la NUpE et la proportion de racines fines seraient des indicateurs de la NUE permettant de cribler précocement des variétés à haut débit.

Title: Identification and genotypic variability of plant traits early determining nitrogen use efficiency (NUE) in winter oilseed rape under low-N inputs

Keywords: Ecophysiology, *Brassica napus* (L.), N-uptake efficiency, genetic variability, proxy-traits, model-assisted phenotyping

Abstract: Improving rapeseed yields in a low-Nitrogen (N) agricultural context is a major issue for breeding. It requires a thorough knowledge of the genotypic variation of the processes related to Nitrogen Use Efficiency (NUE, seed yield per unit of N available). This PhD aims at better understanding the ecophysiological processes determining the NUE and its components under low-N availability by identifying and hierarchizing the main traits supporting observed genotypic variation. Seven winter oilseed rape genotypes were investigated throughout the crop cycle under semi-controlled conditions and contrasting N-conditions. We proposed NUE_DM (plant dry matter per unit of N available), as a new proxy of NUE at harvest, allowing dynamically characterizing NUE in various genotypes from BBCH 59, and in response to N supply from BBCH 30-32.

We highlighted NUpE (plant N-amount per unit of N available) as a main contributor of NUE under low-N conditions, which explained up to 80% of the NUE_DM variations before flowering, and more than 30% after. Moreover, NUpE genotypic variability resulted from fine root growth rather than specific N-uptake differences. We developed a whole-plant conceptual modeling framework of carbon and nitrogen absorption and partitioning for winter oilseed rape. This framework, validated up to flowering, highlighted specific carbon assimilation, carbon partitioning between leaves and stems, and fine root ratio as critical traits explaining contrasting genotypic behavior to N-conditions. Our results suggest NUpE and fine root ratio as promising traits for screening larger sets of varieties for NUE breeding purposes.