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THESE POUR OBTENIR LE GRADE DE DOCTEUR
DE MONTPELLIER SUPAGRO

EN SCIENCES AGRONOMIQUES
ÉCOLE DOCTORALE GAIA
PORTE PAR L'UMR ABSYS ET L'UMR LEPSE
INRAE – MONTPELLIER SUPAGRO

**CHARACTERIZATION OF PULSES FUNCTIONAL DIVERSITY
FOR THE DESIGN OF SUSTAINABLE AGRO-ECOSYSTEMS
UNDER LOW-INPUT CONDITIONS**

PRESENTEE PAR **JULIE GUIGUITANT-PITCHERS**
LE 14 DECEMBRE 2020

SOUS LA DIRECTION DE **DENIS VILE**
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CHARACTERIZATION OF PULSES FUNCTIONAL DIVERSITY FOR THE DESIGN OF SUSTAINABLE AGRO-ECOSYSTEMS UNDER LOW-INPUT CONDITIONS

ABSTRACT

Pulses are generally recognized for their multiple nutritional and environmental benefits. However, most legume species can be defined as orphan crops. This underlines the lack of knowledge on legume diversity and the importance of studying this diversity and elucidating how it supports the functioning of agro-ecosystems. Functional trait approach could guide the inclusion of pulses into agro-ecosystems to provide one or more services. This work aimed at synthesizing current knowledge on the functional diversity of cultivated pulses at the interspecific level as well as producing new insights on the relationships between pulses functional trait variation and agro-ecosystems functioning. We focused on agro-ecosystem properties hypothesized to be good indicators of three specific agro-ecosystem services related to food provision in low-input systems: yield under dry conditions, nitrogen (N) fixation, and competitiveness toward weeds. We combined concepts and methods from functional ecology, crop physiology and agronomy. We applied multivariate statistical methods to data collected from the literature and to data collected from an original field experiment to explore traits-agro-ecosystem properties relationships under multiple environments (literature data) or optimal environmental conditions (field experiment). A controlled experiment in pots allowed to assess the effect of water deficit on key processes such as transpiration and biological N fixation. Our results highlighted covariation patterns and trade-offs between agro-ecological properties supported by different pulses species. Pulses diversity was also described through functional traits and indicated leaf traits as key drivers of inter- and intra-species variability. Nitrogen content of plant organ and seeds traits also captured a great part of pulses diversity. Those traits were related to agro-ecosystem properties. Although they were predicted by multiple alternative combinations of traits it was possible to identify that leaf N content was an important predictor of pulses agro-ecosystem properties. We compared different approaches (meta-analysis and experimental) and discussed intra-species variability. Controlled experiment revealed a large intra and interspecific variability in the response of N fixation and transpiration dynamics, as well as relationships between leaf traits, C/N metabolism, and soil drying-responses of growth, transpiration, and N₂ fixation. We also discussed the intrinsic limitations of trait-based approach and pertinence for its application to agro-ecosystem diversification.

KEYWORD

Diversity, Functional traits, Pulses, Agro-ecosystem, Ecosystem services, Biological nitrogen fixation

CARACTERISATION DE LA DIVERSITE FONCTIONNELLE DES LEGUMINEUSES A GRAINES POUR LA CONCEPTION D'AGRO-ECOSYSTEMES DURABLE EN CONDITION BAS INTRANTS

RESUME

Les légumineuses à graines sont généralement reconnues pour leurs nombreux avantages nutritionnel et environnementaux. Cependant, la plupart de ces espèces peuvent être définies comme des cultures orphelines. Ce constat souligne le manque de données et de connaissances quant à la diversité des légumineuses. L'étude de cette diversité via l'approche des traits fonctionnels pourrait guider l'insertion des légumineuses dans les agro-écosystèmes. Le but de ces travaux a été de synthétiser les connaissances actuelles sur la diversité fonctionnelle des légumineuses cultivées au niveau interspécifique, ainsi que de produire de nouvelles connaissances sur les relations entre les traits fonctionnels et le fonctionnement des agro-écosystèmes. Nous nous sommes concentrés sur un certain nombre de propriétés des agro-écosystèmes dont nous supposons qu'elles sont de bons indicateurs de trois services agroécosystémiques importants et liés à l'approvisionnement alimentaire des système bas intrants : le rendement en conditions sèches, la fixation de l'azote et la compétitivité face aux adventices. Pour cela, nous avons combiné des méthodes et des approches issues de différentes disciplines : écologie fonctionnelle, physiologie et agronomie. Nous avons appliqué des méthodes de statistique multivariée sur une base de données collectées dans la littérature puis aux données originales recueillies lors d'une expérience au champ pour explorer les relations entre traits et propriétés des agro-écosystèmes dans des environnements multiples (données de la littérature) ou dans des conditions environnementales communes et optimales (expérience sur le terrain). Une expérience en conditions contrôlées a également permis d'évaluer l'effet du déficit en eau sur des processus clés tels que la transpiration et la fixation biologique de l'azote. Nos résultats ont mis en évidence la structure de covariation ainsi que des compromis entre les propriétés soutenues par différentes espèces de légumineuses. La diversité des légumineuses a également été décrite par le biais de traits fonctionnels. Les traits foliaires ont été mis en évidence comme étant le principal élément de variabilité inter- et intra-espèces. La teneur en azote des organes végétaux et des semences constituait également un facteur important de la diversité des légumineuses. Ces traits ont été liés aux propriétés des agro-écosystèmes. Bien que ces derniers puissent être prédits par de multiples combinaisons de traits, il a été possible d'identifier le LNC comme un indicateur important des propriétés de l'agro-écosystème. Nous avons comparé différentes approches (méta-analyse et expérimentale) et discuté de la variabilité intra-espèce. L'expérience contrôlée a révélé une grande variabilité intra et interspécifique dans la réponse de la fixation de l'azote et de la dynamique de la transpiration, ainsi que des relations entre traits foliaires, métabolisme C/N et réponses de la croissance, de la transpiration et de la fixation du N₂ au dessèchement du sol. Nous avons également discuté des limites intrinsèques de l'approche basée sur les caractères et de son potentiel pour l'application dans la conception d'agro-écosystèmes diversifiés.

MOTS CLES

Diversité, Traits fonctionnels, Légumineuses, agro-écosystème, Service écosystémiques, Fixation biologique de l'azote atmosphérique

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RESUME ETENDU FRANÇAIS

APPROCHE

Il est reconnu que la biodiversité permet de renforcer la résilience des écosystèmes en favorisant la coexistence de plusieurs espèces qui pourraient remplir des fonctions similaires, mais exprimer des réponses différentes aux perturbations. Un aspect crucial pour le développement de systèmes diversifiés est le choix d'espèces appropriées. L'approche basée sur les traits fonctionnels pourrait guider la conception de mélanges appropriés de variétés et/ou d'espèces pour fournir un ou plusieurs services agro-écologiques. Les légumineuses sont généralement reconnues pour leurs multiples avantages dans les systèmes de culture. Du fait de leur grande diversité, elles présentent une vaste variabilité morphologique et ne sont, de fait, pas équivalentes en termes de fourniture de services écosystémiques. Toutefois, la plupart des espèces de légumineuses peuvent être définies comme des cultures orphelines, c'est-à-dire des cultures marginalisées ou ignorées par les chercheurs, les sélectionneurs et les politiques de développement en raison de leur faible importance économique. Ceci souligne l'insuffisance des connaissances sur la diversité des légumineuses ainsi que l'importance d'étudier cette diversité à travers une approche permettant d'établir des corrélations entre les traits morpho physiologiques de ces espèces et le fonctionnement des agro-écosystèmes et donc plus ou moins directement à la production des services attendus. Ce constat soulève trois questions qui ont guidé les travaux de ma thèse :

- (1) Quels sont les traits structurants la diversité fonctionnelle des légumineuses à graines ?
- (2) Comment la diversité fonctionnelle des légumineuses module-t-elle les propriétés et les services attendus des agro-écosystèmes dans les systèmes de culture ?
- (3) Comment le stress abiotique, en particulier le déficit hydrique, peut-il avoir un impact sur les propriétés des agro-écosystèmes ?

Nous nous sommes appuyés sur le cadre analytique de l'approche basée sur les traits fonctionnels des végétaux. Notre avons fait l'hypothèse que cette approche convient à l'identification de stratégies envisageables pour contrôler conjointement les propriétés multiples des agro-écosystèmes afin d'améliorer la fourniture de services. Nous définissons ici les propriétés des agro-écosystèmes comme des variables ou des processus mesurés ou calculés au niveau des cultures, comme la biomasse ou l'indice de surface foliaire. Les traits fonctionnels sélectionnés sont des caractéristiques d'une plante ou d'un organe végétal, qui peuvent être morphologiques, physiologiques ou phénologiques et qui sont censés avoir des effets directs ou indirects sur les propriétés de l'agro-écosystème. Les services écosystémiques étant encore mal décrits, nous nous sommes concentrés sur les propriétés des agro-écosystèmes, supposés être de bons indicateurs de trois services agro-écosystémiques spécifiques liés à l'approvisionnement alimentaire dans un

système à faible apport d'intrants : 1) le rendement en conditions de limitation en eau ; 2) la fixation de l'azote ; et 3) la compétitivité face aux mauvaises herbes.

Afin de répondre aux questions ci-dessus, cette thèse est organisée en trois sections complémentaires à travers les différentes échelles : (1) une méta-analyse de la diversité fonctionnelle telle que recueillie dans une enquête bibliographique, (2) une étude originale mesurant la diversité fonctionnelle dans des conditions agronomiques plus comparables, permettant une analyse plus précise des compromis entre les traits et les propriétés, et (3) une analyse écophysiological du comportement des légumineuses à graines dans des conditions de déficit hydrique.

RESULTATS

(1) Méta-analyse de la diversité fonctionnelle collectée dans la littérature

Dans une première étude, nous avons cherché à quantifier la diversité fonctionnelle interspécifique des légumineuses en utilisant des analyses en composantes principale (ACP). Les valeurs recueillies pour les six propriétés de l'agro-écosystème ont révélé trois axes principaux de variation dans l'ACP. Le premier axe a classé les légumineuses en fonction de leur niveau de production en termes de biomasse et de grains. Le deuxième axe concernait essentiellement le comportement de compétition, en particulier vis-à-vis des adventices, tandis que le troisième axe classait les légumineuses en fonction de leur capacité à fixer l'azote. Malgré la grande variabilité entre les légumineuses, l'efficacité d'utilisation de l'eau était mal représentée et occupait une position intermédiaire dans cet espace de variation. La structure des co-variations de trait, d'autre part, mettaient en avant la variabilité des traits foliaires et des graines.

Les traits fonctionnels sont directement ou indirectement liés aux processus écologiques qui déterminent les propriétés des agro-écosystèmes. Plusieurs traits peuvent être impliqués dans un processus et un trait peut participer à plusieurs processus. Sur les 6 propriétés des agro-écosystèmes que nous avons étudiées, nous nous sommes d'abord concentrés sur la combinaison de traits qui semblait permettre de prédire l'efficacité d'utilisation de l'eau (WUE). Sur les 28 espèces considérées ici, une WUE élevée a été observée principalement pour les espèces à forte teneur en azote foliaire (LNC). De plus, les espèces à faible LNC étaient plus susceptibles d'avoir une WUE élevée si elles étaient associées à une grande surface foliaire. Le rendement en grains n'a pas été prédit avec le même ensemble de traits que la WUE, à part le LNC. Le poids des graines (TSW) était un bon indicateur du rendement en grains. La régression CART (classification and regression tree) a également montré qu'un taux de fixation élevé était principalement obtenu par des espèces à maturation tardive et a mis en évidence le trait « petites feuilles » comme un trait secondaire impliqué dans la fixation d'azote. Les espèces à biomasse élevée ont quant à elles surtout été associées à une surface spécifique foliaire (SLA) élevée. L'indice de surface foliaire (LAI) était plus élevé pour les espèces à grosses graines à germination hypogée. Enfin, nos résultats montrent que le rendement est

moins susceptible d'être influencé par les mauvaises herbes pour les espèces à maturation précoce et à petites feuilles

(2) Étude de la diversité fonctionnelle des légumineuses à graines, et analyse des compromis entre traits et propriétés en conditions agronomiques similaires

Notre objectif a été de déterminer comment les traits, le rendement et les propriétés connexes de l'agro-écosystème sont reliés entre eux.

Les classements des espèces sur l'axe de variation résumant la variation des caractéristiques architecturales obtenu à partir des données expérimentales, où toutes les données ont été acquises dans un environnement commun, correspondait à celui établi à partir de la méta-analyse de Guiguitant et al. (2020).. Toutefois, le classement sur l'axe résumant la variation de SLA et de LNC étaient différents entre les deux ensembles de données. En outre, les résultats de l'analyse en composantes principales ont montré que la variance totale des traits s'expliquait principalement par la variance entre les espèces.

Les données expérimentales ont confirmé que la longueur des folioles est un élément important de la diversité des légumineuses ainsi que plusieurs autres traits foliaires. La biomasse investie dans les feuilles (LMR), qui contribue à la variabilité totale des traits via le second axe de l'ACP sur les données obtenues dans la même condition expérimentale, était indépendante de l'investissement foliaire en terme de surface (Surface foliaire par plante, LA ; Ratio between leaf area and plant total biomass, LAR). D'autre part, il est apparu que la biomasse investie dans les feuilles (LMR) et la biomasse investie dans les tiges (SMR) étaient corrélées avec des caractéristiques liées au taux de croissance (taux de couverture, MAT). Une allocation plus élevée de biomasse dans les feuilles (LMR) serait liée à une croissance lente tandis que l'optimisation de la surface foliaire (LAR) serait liée une efficacité d'acquisition de ressource. La longueur des feuilles était aussi fortement liée au LNC et, dans une moindre mesure, au SLA. La teneur en azote des graines (SNC) était également corrélée avec le LNC.

Une classification hiérarchique ascendante révéla que les espèces "à haut rendement" avaient une faible capacité de fixation. À l'inverse, les espèces à très faible rendement ont exprimé une augmentation significative de la proportion d'azote d'origine atmosphérique (Ndfa) relative entre la floraison et la maturité. La couverture maximale et la durée de couverture semblaient être liées à la biomasse au moment de floraison et au LAI ainsi qu'au rendement.

Les résultats ont démontré la pertinence de la teneur en azote des feuilles (LNC), du ratio surface foliaire sur biomasse totale (LAR) et de la phénologie pendant la phase reproductive pour prédire la plupart des compromis observés entre les propriétés des agro-écosystèmes. En conclusion, nos résultats mettent en évidence qu'un LNC élevée ne se traduit pas par des rendements plus élevés mais plutôt par une fixation azotée importante. L'investissement du carbone assimilé dans la surface

foliaire (fort LAR) est important pour la fixation de N_2 lors de la floraison, mais il est associé à une sénescence rapide et à un faible rendement. L'investissement en biomasse dans les feuilles et un cycle court coïncident avec des valeurs faibles pour presque toutes les propriétés, à l'exception de la couverture maximale du sol. Enfin, un cycle long et une forte teneur en N à la maturité sont liés à une sénescence tardive et au maintien de la fixation de N_2 au cours du remplissage des gousses.

(3) Analyse écophysiological du comportement de légumineuses à graines dans des conditions de déficit hydrique

Dans cette étude, le seuil de la fraction transpiration de l'eau du sol (FTSW) en dessous duquel le taux de transpiration normalisée (NTR) diminue en réponse au dessèchement du sol a été mesuré pour différentes variétés et espèces du genre *Vigna*. Ce seuil variait entre 0,23 et 0,33. La transpiration de *Vigna* est ainsi apparue moins sensible au dessèchement du sol en comparaison avec d'autres espèces de légumineuses. En outre, certaines des variétés testées présentaient une fixation azotée remarquablement tolérante au déclin de la teneur en eau du sol. Cinq cultivars sur neuf affichaient un seuil FTSW inférieur à 0,11. L'impact de la sécheresse sur le SLA et le LNC a été testé comme une indication des modifications induites par la sécheresse sur la balance métabolique du C et du N. Nous avons observé une augmentation du SLA et du LNC. Par ailleurs, le seuil de FTSW en dessous duquel la fixation azotée diminue en réponse au dessèchement du sol a été aussi mesurée par la méthode de la mesure normalisée de l'activité de l'acétylène réductase (NARA). Ce seuil était plus variable que celui de la transpiration, était compris entre 0,08 et 0,42. Des seuils de FTSW entraînant une baisse de NARA plus élevés étaient associés à une faible augmentation du LNC. Par conséquent, il semble que l'accumulation de N dans les feuilles soit une conséquence du maintien de la fixation de N_2 sur une plus longue période. Ces deux seuils (transpiration et fixation azotée) n'étaient pas corrélés.

Les mêmes mesures ont été réalisées sur 5 variétés de pois chiche (*C. arietinum*). Le seuil de FTSW pour la baisse de la transpiration était en moyenne de 0,54. *C. arietinum* semble donc plus sensible à l'assèchement du sol que *Vigna sp.* D'autre part, le seuil NARA (Normalised Acétylène reductase activity) était compris entre 0,12 et 0,72. La variabilité relative à ce trait était plus marquée qu'au sein des espèces de *Vigna* mais présentait une gamme similaire. En revanche, la plupart des espèces de *C. arietinum* présentaient une sensibilité plus importante que les espèces de *Vigna*. Le seuil NARA ne présentait aucune corrélation avec le seuil NTR. Conformément à *Vigna sp.*, pour la plupart des accessions, la transpiration s'est avérée plus sensible au dessèchement du sol que la fixation azotée. L'ajout des données collectées sur Cicer a. dans l'étude de corrélation précédemment établie sur les *Vigna sp* n'a pas confirmé la relation entre le LNC et le seuil NARA. Malgré tout, le seuil NARA était négativement corrélé au SLA. Cette corrélation n'est pas restée significative lorsque les résultats collectés sur *Vigna* ont été inclus mais a conservé la même tendance. Les variations de SLA et de LNC entre les plantes bien alimentées en eau et celles soumises au manque d'eau s'opposent au

variations observées chez de *Vigna*. En effet, ces deux traits ont montré une augmentation significative lorsque les plantes sont soumises à un déficit hydrique.

Pour conclure, ces résultats sont en accord à une classification des espèces de *Vigna* en tant qu'espèces "acquisitives" et *C. arietinum* pourrait être classé comme espèce "conservatrice" en se basant sur la réponse de leurs traits foliaires au dessèchement et au fait que la transpiration et la fixation azotée de ces deux groupes d'espèces se sont avérées plus ou moins sensibles à l'assèchement du sol.

DISCUSSION

Synergie et compromis entre tolérance au stress hydrique, rendement et services écosystémiques recherchés.

Il est indispensable de comprendre comment les services écosystémiques interagissent pour soutenir le rendement des cultures. Un management orienté vers la maximisation d'un seul service a souvent des impacts négatifs sur les autres et sur la biodiversité (Finney et al. 2017).

Notre étude a permis de constater que les compromis entre les propriétés des agro-écosystèmes des légumineuses étaient relativement cohérents avec la théorie du triangle CSR (Grime et al. 1997 ; espèces Compétitives, Stress-tolérantes et Rudérales) de Grime, qui prévoit que les stratégies végétales sont sélectionnées en fonction de gradients environnementaux de disponibilité des ressources, de stress et de perturbation. D'après la théorie de Grime, ces gradients pourraient avoir optimisé les compromis entre la phénologie ou le développement et l'acquisition de ressources dans les plantes.

Nous avons également analysé plus précisément le caractère antagoniste de certaines propriétés de l'agro-écosystème chez les espèces de légumineuses. Le premier " groupe " de propriétés comprenait les propriétés liées au rendement susceptibles d'être rapportées aux services de production primaire. Un autre ensemble comprenait la biomasse maximale des cultures, le LAI et la couverture du sol, que nous avons associés à la compétitivité contre les adventices dans le premier chapitre. D'autres études ont associé la biomasse des cultures à la prévention du lessivage des nitrates, et la quantité d'azote de la biomasse aérienne, mais elles ont montré qu'elle avait un impact négatif sur la disponibilité de l'azote inorganique et le rendement lors de la saison de culture suivante (Finney, White, and Kaye 2016). Nous avons également identifié un compromis important qui est l'incompatibilité entre un fort rendement et une grande aptitude à fixer l'azote parmi les espèces de légumineuses étudiées. Plus important encore, ces résultats, ainsi que d'autres études, ont mis en évidence le contraste des performances de chaque espèce ou variété et ont souligné que, dès lors qu'ils envisagent d'inclure une culture de légumineuses dans leur système de culture, les agriculteurs doivent avant tout avoir défini leurs objectifs en termes de services. La maîtrise des groupements de services et de leurs compromis peut aider les agriculteurs et les agronomes à concevoir des cultures

diversifiées et multifonctionnelles évitant toute dégradation des services tout en répondant aux principaux objectifs qu'ils se sont fixés.

Il est reconnu également que la biodiversité améliore la résilience des écosystèmes, en permettant la coexistence de plusieurs espèces qui pourraient remplir des fonctions similaires mais qui expriment des réponses différentes aux perturbations (Truchy et al. 2015). Les résultats que nous avons obtenus dans le troisième chapitre ont fait apparaître des comportements spécifiques à chaque espèce dans le cas particulier du dessèchement du sol. Celles-ci pourraient être classées comme "acquisitives" ou "conservatrices" selon la variation de leurs traits foliaires en conditions de stress hydrique. Les résultats ne permettent pas de relier ces processus aux propriétés réelles de l'agro-écosystème, telles que le rendement potentiel ou l'azote maximal fixé. Il n'est donc pas possible de déterminer comment la régulation de ces processus pourrait affecter les propriétés des agro-écosystèmes. Pourtant, en mélangeant des variétés connues pour leur niveau de résistance contrasté à l'assèchement des sols, les agriculteurs seraient en mesure d'atténuer l'impact des perturbations environnementales.

Structures des co-variations de traits et stratégies de production végétale

Les traits des espèces sont un outil efficace pour prédire les effets des changements environnementaux sur la structure des communautés et le fonctionnement des écosystèmes. L'identification préalable des principaux traits clairement associés à la fourniture de services agroécologiques cibles ainsi qu'une large caractérisation de la variation des traits fonctionnels et des compromis entre les espèces et l'environnement permettrait d'améliorer les performances des espèces ou des mélanges de variétés (Lazzaro, Costanzo, and Bàrberi 2018).

Dans le premier et le deuxième chapitre, nous avons montré que les traits foliaires restent un important facteur de variabilité. De manière générale, la taille des plantes cultivées a augmenté depuis leur domestication. D'autres traits susceptibles d'avoir été modifiés par la domestication pourraient être pertinents pour évaluer leur comportement dans les mélanges. Par exemple, l'augmentation de la hauteur du couvert végétal et de la surface foliaire suggère que les espèces cultivées sont de meilleures compétitrices pour la lumière que leurs géniteurs sauvages. L'augmentation de la taille des feuilles au cours de la domestication a été identifiée comme le principal facteur d'augmentation de la taille des plantes. On suppose que la sélection et la domestication ont pu entraîner une réduction de la diversité génétique et pourraient avoir conduit à une uniformité phénotypique (Rubén Milla et al. 2014). Pourtant, les espèces de légumineuses expriment encore une grande variabilité sur ces traits caractéristiques.

Dans le deuxième chapitre, la taille des feuilles a été étroitement associée aux traits liés à l'acquisition de l'azote (LNC, SNC). Tout comme le LNC, les feuilles divisées en petites folioles semblent être une composante fréquente des stratégies écologiques privilégiant un appareil photosynthétique productif. La co-variation du LNC avec le SLA rappelle le 'Leaf Economic Spectrum' (Wright et al.

2004) précédemment décrit et pourrait donc refléter un gradient de la capacité de photosynthèse des feuilles. Cependant, un LNC élevé pourrait avoir d'autres implications biologiques telles que la remobilisation vers les organes reproducteurs (Schiltz et al. 2005). En effet, notre étude a mis en évidence une corrélation entre le SNC et le LNC. Les légumineuses sont également connues pour leurs graines souvent riches en azote par rapport à d'autres espèces de culture. Parallèlement, la taille des graines, représentative d'un deuxième axe de variation entre les espèces de légumineuses, est généralement plus importante chez les espèces cultivées. On suppose que des graines plus grosses pourraient produire des individus avec des organes plus gros par le biais d'effets en cascade (R. Milla and Matesanz 2017). En effet, on s'attend à ce que la taille des graines soit positivement corrélée à la biomasse des jeunes plants, à la hauteur des plantes et à l'effort de reproduction (Tamet et al. 1996).

Les feuilles jouent également un rôle particulièrement crucial dans l'assimilation du carbone, les relations hydrauliques et le bilan énergétique (Ackerly et al. 2002). Par conséquent, la biomasse allouée aux feuilles peut avoir un effet significatif sur l'acquisition des ressources. Ici, l'investissement dans les feuilles (basé sur la surface) semble étroitement lié à l'accumulation d'azote dans la plante et à la réallocation dans les graines. Pourtant, la biomasse investie dans les feuilles (Ratio de masse entre feuille et plante entière, LMR) n'était pas corrélée avec la surface foliaire (LA, LAR). En théorie, l'augmentation du LAR permet d'obtenir une plus grande surface foliaire par unité de biomasse investie. D'autre part, il ressort de nos résultats qu'une allocation préférentielle de biomasse au développement foliaire est liée à une croissance lente.

Nos résultats ont montré que la variance totale des traits s'expliquait principalement par la variance entre les espèces. La variation intra-spécifiques des traits est généralement considérée d'une importance marginale par rapport aux différences interspécifiques. Cependant, les plantes, et parmi elles les légumineuses, présentent souvent une variation intra-spécifique élevée, reflétant à la fois la variabilité génétique et la plasticité phénotypique (Ruelle et al. 2019). Le petit nombre de variétés examinées ici n'a peut-être pas permis de mettre en évidence une telle diversité. Plusieurs études récentes ont montré que la variation intra-spécifique des caractères représente une part importante de la variabilité globale des communautés végétales (Siefert et al. 2015). Nos deux approches (méta-analyse et mesure sur le terrain) ont montré un schéma de co-variation similaire. Ce résultat est conforme à plusieurs études qui ont démontré que malgré la plasticité importante de la plupart des caractères végétaux, la classification des espèces pour un caractère donné reste assez cohérente dans l'espace et le temps. Cependant, les classements sur l'axe résumant la variation du SLA et du LNC ont divergé entre les deux ensembles de données de notre étude. Dans le chapitre 3, nous avons étudié la variabilité intra-espèce de ces traits foliaires ainsi que leur plasticité par rapport à un dessèchement progressif du sol et démontré de forts contrastes entre espèces. Cela suggère que la prise en compte de la plasticité dans l'expression des traits de même que des écarts entre les traits attribués aux espèces coexistantes est essentielle pour élargir les analyses basées sur les traits à la compréhension de

l'assemblage des plantes et de la contribution de chacune des espèces à la structure des communautés végétales.

Des traits aux services agro-écosystème agro-écosystémiques

La recherche agronomique s'est généralement concentrée sur l'utilisation des traits pour comprendre comment la biodiversité dans les systèmes agricoles réagit aux conditions environnementales et à la gestion des cultures, plutôt que sur la compréhension de l'impact de la biodiversité sur les services rendus par les agro-écosystèmes. On observe pourtant un intérêt croissant pour l'utilisation d'approches fonctionnelles pour l'étude et la conception des agro-écosystèmes et la gestion des services agro-écosystémiques.

Les traits foliaires et en particulier le LNC se sont révélés cruciaux dans nos analyses. Soulignant ainsi leur importance pour la prédiction des propriétés de l'agro-écosystème des légumineuses. D'autres caractéristiques telles que le LAR, l'azote total, la taille des graines et la durée du cycle étaient également importantes. Il est difficile cependant de définir une combinaison idéale de traits fonctionnels des plantes qui maximiserait toutes les propriétés de l'écosystème. Nos résultats montrent que le LNC et le PMG (Poids de Mille Graines) permettent de prédire le niveau de rendement. En effet, la taille des graines devrait être positivement corrélée à la biomasse des plantules, à la hauteur de la plante et à l'effort de reproduction. Les résultats du deuxième chapitre ne confirment pas que le LNC joue un rôle dans l'établissement du rendement mais l'associe de préférence à une fixation élevée de l'azote (qui est antagoniste du rendement). Le LNC a également été associé à la WUE, qui est étroitement liée au rendement. En effet, lorsque l'absorption des nutriments est limitée par la disponibilité en eau, la remobilisation de l'azote des tissus végétatifs devient particulièrement importante pour le développement des grains.

En outre, un LNC fort est généralement associée au type « stay green », l'état azoté de la plante est étroitement lié à la longévité des organes photosynthétiques (Borrell, Hammer, and Van Oosterom 2001). Or nos résultats indiquent qu'un cycle long et une forte teneur en azote à la maturité sont tous deux liés à un retard de sénescence et au maintien de la fixation azotée pendant le remplissage des gousses. En outre, une fixation élevée de l'azote est également associée dans nos résultats à des espèces à maturité tardive ou à des feuilles divisées en petites folioles.

Nous avons également montré que les espèces produisant beaucoup de biomasse exprimaient un SLA élevé. Ceci indique un compromis d'efficacité entre carbone investi dans les feuilles et surface foliaire. Or nos résultats montrent également qu'un LAR élevé conjointement à un LMR faible est associé à des espèces pertinentes pour maximiser le deuxième "groupe" de propriétés identifiées (biomasse, LAI, couverture du sol). Nos résultats montrent aussi que la combinaison fort LAR – faible LMR était associée à une faible fixation azotée à la floraison. En effet, la biomasse est difficilement dissociable de la fixation de l'azote car il s'agit d'un processus déterminé par la demande

en azote. Cette "stratégie" d'investissement dans les feuilles a également été associée à une sénescence rapide et à un faible rendement.

Dans notre étude, la réduction de rendement due aux adventices était corrélée avec le LAI, indiquant que la compétition pour la lumière pourrait être un facteur important d'interaction entre les plantes. De nombreuses études écologiques ont souligné que la concurrence s'exprime surtout dans les environnements très productifs. En effet, lorsque les ressources du sol sont plus abondantes, la lumière peut devenir la ressource limitante (Gaudet and Keddy 1988). Dans le premier chapitre, le LAI a été associé aux espèces à grosses graines à germination hypogée, deux traits qui sont généralement associés à une plus forte vigueur de la plantule. En outre, nos résultats montrent que le rendement était moins susceptible d'être affecté par les mauvaises herbes pour les espèces à maturation précoce ayant des feuilles divisées en petites folioles. Les petites feuilles ou les petites folioles sont moins coûteuses à produire et pourraient être plus efficaces pour l'interception de la lumière car elles réduisent le risque d'auto-ombrage (Poorter 1994; Smartt 1978). Outre la taille des folioles, une croissance précoce offre un avantage important à la culture pour l'acquisition de la lumière ainsi que pour l'accumulation d'azote grâce à une croissance racinaire plus rapide et plus profonde (Bedoussac and Justes 2010).

Limites

L'approche suivie dans cette thèse présente certaines limites qui pourraient potentiellement réduire la portée de nos conclusions. Les écologues ont compris depuis longtemps que les différences phénotypiques entre les espèces jouent un rôle important dans la coexistence des espèces. Une écologie végétale "basée sur les traits" fait la promesse d'une prédiction généralisée du fonctionnement et de la structure des communautés indépendamment de la taxonomie. Cette promesse est une raison majeure de la popularité croissante de cette approche. Pourtant, certaines hypothèses fondamentales de l'écologie fondée sur les caractères n'ont pas fait l'objet d'une évaluation empirique suffisante.

Premièrement, le choix des traits qualifiés de "fonctionnels" couramment utilisés dans les études écologiques pourrait ne pas être pertinents pour l'étude des agro-écosystèmes (Jackson 1997). En outre, le champ des combinaisons de traits fonctionnels mesurés sur les plantes est potentiellement vaste. De nombreux traits sont redondants (Laughlin 2014) et il semble y avoir une limite supérieure aux dimensions de la diversité telle que définie par les traits (Lee and Verleysen 2007). En particulier, lorsque l'on considère les espèces de légumineuses, leur capacité inhérente à fixer l'azote est une attente agronomique essentielle. Dans chacun des chapitres de cette thèse, nous avons essayé de mettre en relation les traits morphologiques de la plante avec cette capacité ainsi qu'avec sa régulation en cas de sécheresse. Même si nous avons pu mesurer d'autres caractéristiques dans le chapitre 2, comme le LNC ou la répartition de la biomasse, la variation de la quantité d'azote fixée ne dépend pas seulement de la plante et pourrait être attribuée à des facteurs nutritionnels, aux conditions

environnementales, aux souches de rhizobium ou à d'autres caractéristiques de l'hôte comme les caractéristiques racinaires (Hardarson et al. 1993). Comme les données recueillies dans les différents chapitres de cette thèse portaient uniquement sur les traits aériens des plantes, on a pu obtenir une image incomplète de ce qui détermine l'approvisionnement en azote.

Deuxièmement, malgré le consensus autour de l'intérêt général de la quantification des services écosystémiques (Lavorel and Garnier 2002), il manque des indications systématiques sur les méthodes pour les mesurer et les évaluer quantitativement (Olander et al. 2018). L'existence de nombreuses conceptualisations et systèmes de classification des services écosystémiques a conduit à des différences d'interprétation des services écosystémiques, et de la terminologie qui s'y rapporte lorsqu'il s'agit d'applications (Olander et al. 2018). En outre, les multiples interactions au sein des combinaisons de services n'ont pas fait l'objet d'une grande attention. Pourtant l'impossibilité de gérer les services individuellement nécessite l'aide de modèles pour mettre en œuvre efficacement la prédiction. Dans notre étude, même si nous n'avons pas mesuré directement les services agro-écosystémiques, la mesure sur le terrain des processus sous-jacents (appelés propriétés des agro-écosystèmes) nous a donné un aperçu de ces interactions, même si l'on ne peut pas encore parler de relations quantifiées entre services écosystémiques. Par exemple, l'amélioration de la fertilité de l'azote n'est pas directement liée au pourcentage d'azote atmosphérique fixé et peut dépendre de nombreux autres paramètres.

Troisièmement, la minimisation de la variabilité inhérente à certains traits, tels que le LNC ou le SLA, due à une prise en compte insuffisante de la variation génétique intra-spécifique ou de la plasticité phénotypique constitue une des principales critiques faites aux approches basées sur les traits (Shipley et al. 2016). En supposant que la plupart des traits fonctionnels présentent une forte variabilité intra-spécifique et ont des valeurs qui fluctuent le long de gradients environnementaux, cela ne compromettrait pas nécessairement le fondement théorique de l'écologie basée sur les traits ; cela signifierait seulement que les valeurs des traits devraient être mesurées pour chaque individu plutôt que de se baser sur les valeurs moyennes des espèces ou des sites. L'approche basée sur les traits serait alors plus facile à mettre en œuvre dans les espèces cultivées où l'origine et la génétique des variétés sont souvent mieux décrites et accessibles, par rapport à l'énorme variation génétique rencontrée dans les populations d'espèces spontanées.

Perspectives

Dans l'ensemble, ces travaux visaient à caractériser la diversité fonctionnelle des légumineuses et sa relation avec les propriétés des agro-écosystèmes. Les principales utilisations agronomiques de ces connaissances, en dehors des stratégies de sélection des cultures, seraient d'orienter le choix des cultures pour la diversification des agro-écosystèmes par l'inclusion des légumineuses.

Le choix des espèces et variétés cultivées est figure sans doute parmi les décisions majeures à prendre pour concevoir des agro-écosystèmes plus (bio)diversifiés. Bien que le cadre basé sur les traits ait déjà été largement appliqué aux agro-écosystèmes, la plupart de ces recherches ont concerné les pollinisateurs, les adventices ou les micro ou macro-organismes du sol. Les recherches sur la contribution des cultures à la productivité se sont largement concentrées sur les classifications des groupes fonctionnels. Cependant, certaines contraintes peuvent provenir de la complexité des processus écologiques dans les assemblages d'espèces. Par exemple, dans les mélanges variétaux, la réponse d'une variété donnée peut varier en fonction de l'autre variété du mélange et cette réponse plastique peut influencer de manière significative la productivité desdits mélanges. Par conséquent, les traits mesurés dans des peuplements monospécifiques peuvent ne pas être suffisants pour mettre en place des systèmes de culture efficaces, stables et prévisibles.

Pourtant, l'approche fondée sur les traits, combinée au concept de niche pourrait guider la conception de mélanges appropriés de variétés et/ou d'espèces pour fournir de multiples services agro-écologiques (Wendling et al. 2017; McGill et al. 2006). Les études ont montré que les différences de traits génèrent des hiérarchies dans les aptitudes à la compétition. La grande variation des valeurs des traits que l'on trouve dans la plupart des communautés et des études montrant que les valeurs des traits des espèces cooccurrentes sont très différentes est la preuve que les traits jouent un rôle dans le maintien de la diversité des espèces par le cloisonnement des niches. Bien que la littérature, mettant en corrélation les performances phénotypiques et les capacités compétitives ait historiquement mis l'accent sur la stabilisation de la différenciation des niches, toutes les différences phénotypiques ne sont pas propices à la coexistence, ce qui complique les efforts visant à prédire l'assemblage des communautés à partir des modèles de traits. Pourtant, une telle approche appliquée à l'agronomie offrirait la possibilité de comprendre les règles d'associations pour la conception d'agro-écosystèmes diversifiés sans avoir à tester chaque association envisagée une à une, économisant ainsi bon nombre d'expérimentations difficiles à interpréter et peu généralisables.

CONCLUSION

Notre travail a contribué à l'élaboration des connaissances nécessaires pour l'application du cadre analytique basé sur les traits fonctionnels appliqué à la diversification des systèmes de culture utilisant des légumineuses à graines. Cela a été possible grâce à une large caractérisation de la variabilité des traits fonctionnels des légumineuses à graines, l'identification des principaux traits déterminants pour la prévision des propriétés des agro-écosystèmes, et une analyse de la réponse physiologique de la fixation de l'azote et de la transpiration aux conditions environnementales. Ce travail contribue à la définition d'une "niche" basée sur les traits qui s'alignerait sur les stratégies d'acquisition des ressources et affinerait notre compréhension de la réponse des agro-écosystèmes aux perturbations environnementales.

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CHAPTER 1: *GENERAL INTRODUCTION*

A. Diversity to support resilience and ecosystem services

Today's agriculture have to meet environmental challenges while coping with climate change, growing population and depletion of resource for agriculture (Godfray *et al.*, 2010; Massawe, Mayes and Cheng, 2016). Besides, one of today's most urgent challenges faced by society is slowing down global biodiversity loss (Roe, 2019). The recognition of biodiversity as an inherent component of ecosystem sustainability (Walker, 1995; Yachi and Loreau, 1999) points out to the utility of crop diversification for maintaining agro-ecosystems services. Agro-ecosystems services are externalities of the agro-ecosystem that confers direct or indirect benefits to human beings. Among the variety of crops that can be used, pulses (crop with edible seeds of plants in the legume family) afford interesting agronomic, environmental, and nutritional benefits (Voisin *et al.*, 2014). Grain legumes display a wide morphological diversity (Doyle, 2003; Edwards, 2007) that can result in various ecosystem services. This diversity, if explored and characterized, could provide solutions for localized adverse conditions and participate in the production of various services expected from legume crops.

I. Biodiversity in agro-ecosystem

Biodiversity, by allowing the co-existence of several species which could fulfill similar functions, but express different responses to perturbations, is recognized to enhance ecosystem resilience (Walker, 1995; Yachi and Loreau, 1999). Although the idea of building resilience has been studied in a broad range of ecosystems, from coral reefs to forests (McCook *et al.*, 2007; Thompson *et al.*, 2009; Truchy *et al.*, 2015), this idea has been scarcely studied in agro-ecosystems (Lin, 2011; Cabell and Oelofse, 2012; Altieri *et al.*, 2015).

Agro-ecosystems exist by and for human-being and are managed with a clear goal: produce food, feed or other goods for the household use and/or for the market. They are defined as cultivated ecosystems and are generally characterized by (Jensen, Peoples and Hauggaard-Nielsen, 2010) :

- (1) A spatial delimitation which comprises a system of crops and pastures in interaction with a biological, chemical and physical environment, generally defined at plot, farm or landscape scale. It seems that farm-scale is a crucial level to study resilience of ecosystem services to biotic and abiotic perturbations (Reidsma, Ewert and Oude Lansink, 2007; Teixeira *et al.*, 2018);
- (2) A temporal delimitation defined by succession of crops and pastures in the system,
- (3) A management strategy, including the selection of pertinent species and varieties.

As an ecosystem managed to benefit human-being, most outputs derived from an agro-ecosystem are referred to as 'ecosystem services'(Costanza *et al.*, 1997). The difference with natural ecosystems is

that ecosystem services provided by an agro-ecosystem are primarily services that support the primary production processes.

Biodiversity is assumed to increase ecosystem functioning in natural ecosystems. Indeed, both ecology and agriculture confirm that a greater genetic diversity makes a system more resilient to environmental changes such as rainfall and temperature fluctuations (Falco and Perrings, 2003). The objectives of biodiversity conservation in agro-ecosystems are strongly related to the farmers goals in terms of production, and economic and environmental considerations (Tanaka et al., 2002). Agricultural systems offer a large range of diversification possibilities giving farmers a wide variety of options and combinations for the implementation of this strategy. Diversification can occur in many forms (genetic variety, species, structural) and over different scales (field, farm, landscape). The comparative benefits of enhanced crop diversity are mainly supported by the complementary use of resources temporally (Yu et al., 2016; Dong et al., 2018), spatially (Corre-Hellou et al., 2007; Bedoussac and Justes, 2010), or through differentiated physiological functioning that enables to rely on distinct resource pools. This complementarity of resource use by diverse plants enhances resource capture and resource use efficiency at the scale of the agro-ecosystem (Li et al., 2014).

II. Design and evaluation of crop diversification strategies

Despite several attempts made to translate ecological findings into agronomic innovations, designing crop mixtures is still a challenge for agronomists, physiologists and plant breeders. Methods are still needed for the design and evaluation of diversified cropping systems (Groot, Oomen and Rossing, 2012). The effect of crop diversity on productivity is easily assessed for rotation as a simple measure of the yield obtained from the crop of interest in rotation compared with a base-line (generally monoculture) will give a satisfactory information. Similarly, the land equivalent ratio (LER) provides a standardized basis to assess mean production of intercropping systems. However, the effect of biodiversity covers more dimensions than yield increase or decrease. For example, these two metrics do not account for the risk-reduction effect obtained by diversification (Paut, Sabatier and Tchamitchian, 2020). Theories derived from economic studies have made it possible to formalize the risk reduction effect of crop diversification and choose an appropriate diversification design to increase yield and reduce yield variability (Modern Portfolio theory; Paut, Sabatier and Tchamitchian, 2019). The Modern Portfolio theory relies on the use of a mathematical model combining both yield and yield variability (standard deviation), but also meets limitations. The model needs to be fed with two or more crops and need an estimate of yield covariation for these crops. Therefore, it requires considerable long term data to produce robust estimates of yield covariations. In addition, it doesn't take into account interactions when crops are mixed together. Another issue is that most of the data available in the literature focus on the performance of a variety or a species sown as a sole crop, which is often not sufficient to predict its behavior in a complex cropping system (Lithourgidis *et al.*, 2011).

There is still a need for an efficient and simple framework/model to capture complementarities in crop behavior and design diversification strategies. Diversification evaluations relying on botanical or taxonomic aspects (as proposed by Figge, 2004) does not seem helpful to capture complementarities in crop behavior (Paut, Sabatier and Tchamitchian, 2020). Niche complementarity, on the other hand, is a key ecological theory suggested to describe this interaction in crop mixtures (Wendling et al., 2017). Three axes can be used to illustrate species niches (Chase and Leibold, 2003): resources, biotic enemies and abiotic stresses. These axes are theoretically associated with sets of phenotypic traits. Most research relative to the impact of biodiversity on ecosystem up to date were based on studies manipulating species richness (defined as the number of different species represented in a community) but there is growing evidence that functional diversity (the diversity of species' niches or functions) of a community is often more important to ecosystem functioning than richness (Cadotte et al., 2009; Flynn et al., 2009; Mouillot et al., 2011; Finney and Kaye, 2017). Such findings have significant implications for agro-ecosystems; optimizing diversity for ecosystem services may not require maximizing species or varieties number. Rather, farmers could design polycultures that provide desired services using a selected reduced set of species combinations that augment biodiversity while minimizing economic or management constraints (Finney and Kaye, 2017)

III. Trait-based approach for mixture design

A crucial aspect for further development in intercropping systems is the choice of suitable species (Brooker et al., 2015). To address this concern, two non-exclusive strategies can be proposed to detect or predict the best combinations of varieties (Barot et al., 2017). The first one is a "blind to trait" screening of a wide range of randomly assembled species or varieties. This approach was first developed for cultivar mixtures (Lopez and Mundt, 2000; Dawson and Goldringer, 2011) but can be expanded directly to intercropping and temporal successions of crops. Although this approach may be attractive, it has a major limitation: it requires many midterm field experiments with several replicates which can be difficult to implement. The second approach is "trait-based": the species/varieties assembly are obtained based on the selection of a combination of plant functional traits that would maximize targeted agro-ecological services. Functional traits are morphological, ecophysiological or phenological characteristics of plant species or varieties that can influence ecosystem or agro-ecosystem functions and, ultimately, the provision of services (Lavorel and Garnier, 2002; Violle *et al.*, 2007; Garnier and Navas, 2012). This latest approach could guide the conception of appropriate mixtures of varieties and/or species to provide single or multiple agro-ecological services (Litrico and Violle, 2015; Martin and Isaac, 2015a; Wood *et al.*, 2015a). In this case, a set of varieties characterized for their functional characteristics can be chosen to maximize the set of values for relevant traits.

Species traits offer a potentially powerful tool for predicting the effects of environmental changes on community structure and ecosystem functioning and are a key component in the further development and application in the context of ecosystem services. The study of traits is also at the heart of physiology, it is even central in mechanistic physiology (Chown, 2012). The approach is therefore common to both disciplines (ecology, physiology) but is deployed at different scales within each of them. The focus of ecology is on regulation and interactions within populations and communities, while that of physiology is generally on plant-organ scales (McNab, 2002; Chown and Storey, 2006). For a better understanding of the functioning of an organism in a given environment and the effect of these variations in this functioning on the fitness of an individual and impacting the performance of an ecosystem, it seems interesting to integrate complementary approaches based on traits.

To date, robust species trait databases are a limiting factor for developing a trait-based approach aimed at maximizing agro-ecosystem services (Barot et al., 2017). Furthermore, most of these databases are static, with a limited coverage of intraspecific variations within populations or species' range (Truchy *et al.*, 2015). Developing objective trait classifications across a broad range of groups requires important and integrated research efforts in various disciplines. Furthermore, this research should focus on which processes/supporting service measures, and possibly ecosystem functioning "multimetrics", work best as proxies for final ecosystem services, across a range of environmental and socio-economic contexts (Truchy et al., 2015).

B. Legumes: a crucial role to play in the diversification of cropping system

I. An underused diversity

Many grain legumes are traditionally cultivated and maintained worldwide resulting in the evolution of a wide genetic diversity. This diversity is poorly represented in most cropping systems despite increasing demand from food chains (De Boer and Aiking, 2018). Most legume species could be defined as orphan crops (Cuevas, 1992; Foyer *et al.*, 2016), i.e. crops that are marginalized or ignored by researchers, breeders (Varshney *et al.*, 2009) and development policies due to their lack of economic importance (Padulosi, Thompson and Rudebjer, 2013; Foyer *et al.*, 2016; Cullis and Kunert, 2017). They are, for the most part, locally important crops maintained by cultural preferences and traditional practices because they are nutritionally rich (Padulosi, Eyzaguirre and Hodgkin, 1999; Ghane *et al.*, 2010) and adapted to difficult environments where they can still provide a sustainable production unlike cereal crops (Mal, 2007). This lack of attention creates a risk of erosion of genetic resources and could lead to a severe restriction of development options for food security (Dansi *et al.*, 2012). While more than 80 species of pulses are actually consumed worldwide, the FAO database

covers only 11 of these species (Tiwari, Gowen and McKenna, 2011). The number of cultivars in circulation is also very low: in France, for example, only 7 chickpea cultivars are available on the market, while more than 90,000 accessions are recorded in the world's genetic resources (Varshney, Thudi and Muehlbauer, 2017). Constraints preventing more widespread cultivation and use of pulses could be attributed to their low importance within food systems and their limited market opportunities (Magrini *et al.*, 2016).

Genetic diversity is essential for the provision of ecosystem services in agro-ecosystems (Hajjar, Jarvis and Gemmill-Herren, 2008). Advances in genomics and phenotyping technologies, as well as the recognition of their importance for the ecology of both natural or agronomic systems, are gradually gaining ground. In the recent years, many legume crops genetic systems have been described. However, comprehensive genetic analysis is limited to a few cultivated legumes, due to the large size of their genomes. For soybeans, the most widely grown and economically important legume, the full genome has been sequenced from 2010 (Schmutz *et al.*, 2010). For the common bean (*Phaseolus vulgaris*), the most widely grown grain legume, the full genome was sequenced in 2014 (Schmutz *et al.*, 2014). Many more legume species have been sequenced since then. Despite their close phylogenetic relationships, legume crops differ greatly in their genome size, base chromosome number, ploidy level, and reproductive biology (Smýkal, von Wettberg and McPhee, 2020). Although Mendel's pea was the first "model" plant, legume biology has long lagged behind more successful or economically important models. Pulses have been the "slow runners" of the green revolution due to small genetic gains in comparison with those of cereal breeding over the last century. However, addressing knowledge gaps in pulse genetics is essential (Sahruzaini *et al.*, 2020).

II. Context in Europe

Compared with other major agricultural regions of the world, the European Union (EU) is characterized by a low level of vegetal protein production (Roman *et al.*, 2016). Moreover, pulses cropping area have been declining on a yearly basis from 1961 (4.8% of EU arable land), until 2013 (1.1% of EU arable land), (FAO, 2020). Dry pulses have frequently been substituted by cropping alternatives with better gross margins (Preissel *et al.*, 2015) and more stable yields (Reckling *et al.*, 2015; Cernay, Pelzer and Makowski, 2016). This trend is attributable to the historic signature of trade agreements between Europe and the United states: European and French public institutions decided to implement cereal price support, committing in return to allow the entry of unlimited quantities of oilseeds without customs duties (Thomas, Schneider and Pilorgé, 2013; Magrini *et al.*, 2016). However, other evolutions in breeding selection, public subsidies, and food systems, have also favored the development of cereals to the detriment of grain legumes (Magrini *et al.*, 2016). However, renewed interest for legumes emerged in Europe over the past decade and the share of legumes in the cropping area in Europe went up raised from 1.1 to 2.2% in 2015 (FAO, 2020). In 2014, after the most recent CAP reform, 30% of pillar I direct payment became conditional on so-called greening

measures: crop diversification (for larger arable farms); maintenance of permanent grassland; and maintaining so-called ecological focus areas (EFAs) on 5% of farmland, later to be expanded to 7%. Both the crop diversification requirement and the EFA may have favored legume share in cropping systems (Kuhlman, Helming and Linderhof, 2017; IPBES, 2019). Furthermore, in France, the recent Senate report " Vers une alimentation durable: Un enjeu sanitaire, social, territorial et environnemental majeur pour la France " has identified legumes as an ecological and economic issue, a keystone of food system evolution; thereby confirming an already established intention to develop the use of legumes over the past few years.

III. Properties of legumes and expected benefit for agro-ecosystems

1. *Agro-ecosystem productivity*

Legumes are usually praised for their multifaceted advantages (Jensen, Peoples and Hauggaard-Nielsen, 2010; Voisin *et al.*, 2014). Within the developing world, dietary protein sources are often limited to cereals due to higher prices of meat and the amount of protein consumed is often insufficient in comparison with dietary requirements (Henchion *et al.*, 2017). Pulse crops are among the best plant-based sources of dietary protein and other nutrients such as iron and of dietary fiber (Kouris-Blazos and Belski, 2016). They contain high levels of globulins and albumins, providing around 33% of dietary needs (Bessada, Barreira and Oliveira, 2019). Thus they provide an affordable alternative source of proteins (Singh *et al.*, 2003; Tharanathan and Mahadevamma, 2003). Moreover, given the fact that they can be used for feed and food they are suitable crop alternative in many agro-ecosystems (Peltonen-Sainio and Niemi, 2012). As already mentioned, crop diversification takes many forms which could be defined as a gradient of temporal (crop sequences) and spatial (intercropping) diversification schemes. Legumes are already used for crop diversification in many systems and have proven their value. Forage cropping systems with legumes usually have higher or equivalent gross margins (Reckling *et al.*, 2016). When considering arable lands, the economic argument is less obvious. Recent study showed a crop rotation including pulses may provide higher net return than wheat monoculture (Khakbazan *et al.*, 2020), but when comparing with non-legume crop sequences (e.g. rapeseed-cereals rotation), gross margins are generally lower (Reckling *et al.*, 2016). To assess the farm economic value of pulses, their contribution to the yield variation of the following crops in agro-ecosystem must be taken into account (pre-crop value ; Preissel *et al.*, 2015). Alternatively, legumes can be introduced in agro-ecosystem through intercropping, which is an ancient practice. Legume-cereals intercropping is widely used in many countries such as Latin America (Paudel, 2016); rural sub-Saharan Africa (Matusso, Mugwe and Mucheru-Muna, 2014), or China (Gong *et al.*, 2019). The maize-soybean intercropping system has become increasingly popular in many areas of the world, particularly in China, due to its high productivity and the difference in

shape, size and color of the two grains that can be easily machine harvested and sorted (Liu *et al.*, 2017).

Yield increase of subsequent or adjacent crops are the major benefit of pre-crop value (defined as the benefit of a previous crop for a subsequent crop in crop sequencing) involved in rotation and complementarity/facilitation process of intercropping. It is well-known that including legumes in a crop sequence generally improves yield and yield stability of the following crops (Evans *et al.*, 1991; Jensen *et al.*, 2004; Nemecek *et al.*, 2008; Peoples *et al.*, 2009; Yu, Xue and Yang, 2014; Angus *et al.*, 2015; Preissel *et al.*, 2015; Fan *et al.*, 2020; Liu *et al.*, 2020, 2020). Similar conclusions have been raised for intercropping system including pulses (Pitan and Odebiyi, 2001; Mpairwe *et al.*, 2002; Dapaah *et al.*, 2003; Bedoussac *et al.*, 2015; Carton *et al.*, 2020). Legume-cereal intercropping systems would enhance complementarity/facilitation processes, thus improving resource utilization, leading to an increase in crop production corresponding to greater efficiency of the agro-ecosystem in general (Duchene, Vian and Celette, 2017). The role of grain legumes in cropping systems (either intercropping or rotation) is increasingly well understood with respect to N fixation, mechanisms of pre-crop effects, and environmental impacts (Nemecek *et al.*, 2008). But experimental results on benefits magnitude vary widely and require distinction between climatic regions, N fertilizer intensity, and compared crop to break disease, pest and weed cycles and optimize nutrient management (Stagnari *et al.*, 2017).

2. *Improvement of crop nitrogen nutrition*

Legumes are usually included in cropping systems with the expectation that they will provide nitrogen to the system that will benefit the principal crop. Indeed, N fertility, obtained from either fertilizer or legumes in monoculture or rotation systems, is probably one of the most, if not the most important leverage to reduce yield variability (Varvel, 2000). Many studies have quantified the pre-crop value of legume species in terms of fertilizers reduction while introducing a legume prior to cereal cultivation. Legume introduction in the rotation could reduce the economic optimum N application rate for cereal crops up to 43–153 kg fertilizer-N ha⁻¹ (St. Luce *et al.*, 2015; Oliveira *et al.*, 2019). Similar results can be found in intercropping. Cereal-pea intercropping can improve grain and straw quality of the intercropped cereal and allow reduced N inputs compared with sole cropping (Lauk and Lauk, 2008; Yu, Xue and Yang, 2014; Monti *et al.*, 2016; Kakraliya *et al.*, 2018). Although both types of diversification could improve N balance, the mechanisms supporting this apparent benefit seem rather different.

The positive response of the cereal in rotation with legumes is attributed to increased levels of readily available nitrogen. Some studies have observed an increase in mineral N in root-zone of cereal preceded by a legume compared to cereal in monoculture (Dalal *et al.*, 1998; Jensen *et al.*, 2004; López-Bellido, Muñoz-Romero and López-Bellido, 2013; Plaza-Bonilla *et al.*, 2016; Peoples *et al.*,

2017). Increase in soil N also occurs for non-legume preceding crop but was found greater from legumes than non-legume crop (Iannetta *et al.*, 2016). Incremental changes in soil N associated with the pulse crops were found strongly correlated to N₂ fixation and were highly variable, particularly when a cropping system is evaluated over a long term (Walley *et al.*, 2007; Iannetta *et al.*, 2016). Nitrogen mineralized from grain legume residues was estimated to contribute to only 15-40% of N acquired by the following wheat crop (Evans *et al.*, 2001). The remaining percentage of cereal N must be obtained from pre-existing soil sources. Net soil N balances in grain legume-cereal rotations may be positive or negative depending on the legume species (Lauk and Lauk, 2008; St. Luce *et al.*, 2015; Guinet *et al.*, 2019), symbiotic performance, and agronomic factors (such as crop residues management) (Chalk, 1998; Walley *et al.*, 2007). For example, pulse crops that typically achieve only modest levels of N₂ fixation such as *Desi* and *Kabuli* chickpea or common bean are more likely to be either N neutral or contribute to a soil N deficit (Walley *et al.*, 2007). The management of legume stubble and straws (exported, buried, or let on the surface of the soil) is also, obviously, a major determinant of the contribution of legumes to N soil balance. Moreover, when management of N balance is not well understood, incorporation of legume in a system could lead to nitrate leaching (Hauggaard-Nielsen, Mundus and Jensen, 2009; Plaza-Bonilla *et al.*, 2016).

In intercropping, any species utilizing the same combination of resources will be in direct competition. However, based on differences in phenological characteristics of species in mixtures, the interaction among them may lead to an increased capture of a limiting growth resource (Willey, 1990). Most annual crop mixtures such as those involving cereals and legumes are grown almost at the same period, and develop root systems that explore the same soil zone for resources (Ndakidemi, 2006). Thus even if they might have slight N transfer through mycorrhizal interaction (Šarūnaitė, Deveikytė and Kadžiulienė, 2010), improvement of the N nutrition of the overall cropping system is mainly due to the ability of the grain legume to rely on another source of N. Results highlighted that intercropping consistently stimulates complementary N use between legumes and cereals by increasing N₂ fixation by grain legumes and increasing soil N acquisition in cereals (Rodriguez *et al.*, 2020).

3. *Improvement of soil properties*

While some of legume's rotational advantages may be associated with improvements of the availability of N in soils, factors unrelated or indirectly related to N also play an important role (Osterholz, Liebman and Castellano, 2018).

High phosphorus (P) availability presumably improves biomass and grain yield in intercropping (Latati *et al.*, 2016). Excessive applications of P fertilizers and P-rich manures have led to an accumulation of P in many soils, which lowers P-fertilizer-use efficiency and leads to P losses via runoff and eutrophication of surface waters (Zhang, Chen and Vitousek, 2013). Increased P uptake in

intercropping can be achieved through complementarity or facilitation interaction (Xue *et al.*, 2016). Niche complementarity can occur when two intercropped species tap into different pools of soil P, such as inorganic and organic pools (Li *et al.*, 2008) or different chemical forms of each of these pools (Wang, Marschner and Zhang, 2012). Interspecific facilitation in P uptake occurs when one species increases soil P availability and the intercropped companion species can take advantage of that. Intercropped cereals benefit from legumes in terms of P acquisition mostly in alkaline and neutral soils in which rhizosphere acidification in response to N₂ fixation raises P availability (Xue *et al.*, 2016). Most cereal/legume intercropping studies implicitly assume that cereals will benefit from the legumes because the roots of legumes are known to secrete larger amounts of protons (Tang, Barton and McLay, 1997), carboxylates (Pearse *et al.*, 2006) and phosphatases (Makoi, Chimphango and Dakora, 2010) per unit of rhizosphere soil than those of cereals.

Moreover, the production of hydrogen gas (H₂) as a by-product of nitrogen fixation have recently been shown to impact significantly microbial communities. Legume symbioses with rhizobia lacking hydrogenase enzymes (which can recycle hydrogen) have traditionally been viewed as energetically inefficient. However, recent studies suggest that hydrogen release to soil may be beneficial to increasing soil carbon sequestration and promoting growth of hydrogen-oxidizing bacteria beneficial to plant growth both in intercropping and rotation (Golding, 2009; Peoples *et al.*, 2009; Golding and Dong, 2010; Angus *et al.*, 2015)

A higher crop diversity would also result in higher soil microbial biomass and richness (Bartelt-Ryser *et al.*, 2005; Yu, Xue and Yang, 2014; Venter, Jacobs and Hawkins, 2016; Qin *et al.*, 2017; Borase *et al.*, 2020). Change in microbial population may induce differences in the catabolic capability of soil (Aschi *et al.*, 2017; Borase *et al.*, 2020). Legumes also seem to induce a change in the surrounding habitat of microbial communities by increasing the active (labile) carbon pool (Hernanz, Sánchez-Girón and Navarrete, 2009; Nath *et al.*, 2019; Sánchez-Navarro *et al.*, 2019) and achieving a suitable soil pH (Qin *et al.*, 2017; Borase *et al.*, 2020).

The benefits of intercropping are also highly subjected to microbial communities and particularly to the mycorrhizal symbiosis establishment and its impact on the soil microflora functionalities (Wahbi, Prin, *et al.*, 2016). In particular, it is well known that plant species, highly dependent on the mycorrhizal symbiosis for their growth (i.e., legumes) will promote the development of the mycorrhizal fungal growth (Duponnois, Plenchette and Bâ, 2001; Ingraffia *et al.*, 2019). Mycorrhizal fungi facilitate the uptake and the transport of less mobile soil nutrients (such as P) (Tang *et al.*, 2014) by plants, enhance N fixation and drive biological interactions among neighboring plants (Ingraffia *et al.*, 2019).

Finally, in the short term, plant diversity effects, including legume diversity effects on soil are mediated by a general stimulation of soil microbes and nutrient availability which mostly beneficiate

intercropping whereas the longer-term effects of particular plant species are more likely due to compositional shifts in soil microbial communities (Bartelt-Ryser *et al.*, 2005).

4. *Weed, pest and auxiliaries*

Among the advantage of crop diversification with legumes, a lot of biotic interactions between the different system components are expected. Weed and pest control is often referred to as an important concern, especially when farmers are aiming at reducing phytosanitary inputs. It has been highlighted that direct weed control can only be successful where preventive and cultural weed management is applied to reduce weed emergence (e.g. through appropriate choice of crop sequence, tillage, cover crops) (Bàrberi, 2002). As a consequence, weed management must be approached on a long-term basis and requires an integration of many cultural practices. Pulses, as break-crop or as part of a rotation, can contribute to weed or pest control (Seymour *et al.*, 2012). They represent a source of diversification in order to break disease, pest and weed cycles and optimize nutrient management in standard crop rotations (Stagnari *et al.*, 2017). Weed biomass (Midya *et al.*, 2005; Banik *et al.*, 2006) and pest abundance (Wahbi, Maghraoui, *et al.*, 2016) can also be significantly reduced in cereal-legume intercropping systems compared with pure stands. However, crop species and varieties express various shading ability, and therefore different competitiveness against weeds (Gollner, Starz and Friedel, 2019).

Another biotic interaction which have received less attention is the impact of legume usage on pollinators. Legumes are also known as bee-pollinated species (Marzinzig *et al.*, 2018). The importance of plant-pollinator interaction in flowering crops such as legumes is based both on the dual roles of pollination for production services and breeding strategies, as well as the increasing need to mitigate the decline of pollinators. Pollinator-friendly legumes can significantly contribute to the habitat zone. Due to their specific flower type and the unusual color of the petals, they can attract, for example, wild bees, which are also effective for many other crops (Suso *et al.*, 2016). Among strategies generally proposed in favor of pollinators, flowering crops are much more appreciated by farmers than flower strips or other unproductive habitats (Suso *et al.*, 2016).

5. *Effect on climate change mitigation*

Among their many significant benefits for society, pulses' role in alleviating climate change has rarely been discussed. Pulses could (1) reduce emissions of greenhouse gases such as carbon dioxide (CO₂) and nitrous oxide (N₂O) compared to agricultural systems based on N mineral fertilization, (2) play an important role in soil carbon sequestration, and (3) reduce overall fossil energy inputs to the system.

Estimations of the final greenhouse emission outcomes of introducing pulses into cereal-based cropping systems are still uncertain, but current information suggests that replacing a cereal with a

pulse crop will likely result in no change or a small but significant decrease of emissions to the atmosphere (Lemke *et al.*, 2007). N₂O represents about 5–6% of the total atmospheric greenhouse emission, but it is much more active than CO₂ (Crutzen *et al.*, 2007). Agriculture represents the main source of anthropogenic N₂O emissions (about 60%; a majority of these emissions results from the application of nitrogen fertilizers (Reay *et al.*, 2012)). Including one legume crop in a cereal rotation based on mineral fertilization may decrease N₂O emissions by 20–25 % (Jeuffroy *et al.*, 2013; Reckling *et al.*, 2016)

Nevertheless, it is important to highlight that the influence of legumes in reducing greenhouse emission depends also on the management of agro-ecosystems in which they are included and may lead to higher cumulative N₂O emissions (Senbayram *et al.*, 2016). A study suggested that legumes have increased up soil organic carbon (SOC) to 30% when included in rotation as compared with monocropping ; this would be due to their capacity for nitrogen fixation (Tiemann *et al.*, 2015). The potential for C sequestration and the amount of organic C returned to the soil by legume species depends largely on the legume species, their growth behavior, root and leaf morphology and physiology, as well as on crop management and growing conditions (Kumar *et al.*, 2018; Hazra *et al.*, 2019).

C. Problematic and approach of the thesis

I. Research questions and general methodology

In the previous section, we underlined the insufficient knowledge on the diversity of pulses as well as the importance to study this diversity through an approach enabling the correlation with agro-ecosystem functioning and thus more or less directly to the provision of expected services. This raised three questions which have guided my thesis' work:

- (1) Which traits are structuring pulses species functional diversity?
- (2) How does pulses functional diversity modulate the agro-ecosystem properties and services expected in cropping systems?
- (3) How does abiotic stress, in particular water deficit, would impact agro-ecosystem properties?

This work aimed at synthetizing current knowledge on the functional diversity of cultivated pulses at the interspecific level as well as producing new insight on the relationships between pulses functional trait variation and agro-ecosystem functioning. We used a trait-based analytic framework as defined earlier (Garnier and Navas, 2012; Martin and Isaac, 2015b; Wood *et al.*, 2015b). We assumed that this approach is appropriate to identify possible strategies by which multiple agro-ecosystem properties can be jointly controlled for a better ecosystem services provision. Here we define agro-

ecosystem properties as variables or processes measured or calculated at the crop level (Garnier *et al.*, 2007), such as biomass or the leaf area index. Selected functional traits *sensu* Violle *et al.* (Violle *et al.*, 2007) are characteristics of an individual plant or plant organ, which can be morphological, physiological or phenological and that are supposed to have direct or indirect effects on agro-ecosystem properties. Since ecosystem services are still poorly described, we focused on agro-ecosystem properties hypothesized to be good indicators of three specific agro-ecosystem services related to food provision in a low-input system: 1) yield under dry conditions; 2) nitrogen fixation; and 3) competitiveness toward weeds.

II. Hypotheses

Based on current knowledge in ecology and agronomy, we elaborated several hypotheses related to the functional diversity of pulses species.

Hypothesis 1: pulses functional diversity, as indicated by individual plant traits is a determinant of the variability of properties observed at the canopy or plant stand scale.

Hypothesis 2: all agro-ecosystem properties (production, soil cover, nitrogen fixation, transpiration, etc.) cannot be jointly maximized in space and time, therefore trade-offs between properties are expected in relation to variation of specific functional traits combinations.

Hypothesis 3: within-species variability, although lower than between-species variability, is of significant importance and trait-properties relations vary across environments and when subject to abiotic stress.

Hypothesis 4: The diversity of pulses can be used for agro-ecosystem diversification and can impact agro-ecosystem services provision. Functional traits offer a possibility to guide the choice of appropriate species/varieties.

III. Approach

To analyze the functional diversity of grain legumes and discuss its impact on the services expected within an agro-ecosystem, we have chosen an integrative approach, combining three disciplines (Figure 1): functional ecology, which defines diversity as a set of traits whose quality and relative abundance are linked according to a number of hypotheses to the properties of the ecosystem; plant ecophysiology, which aims at modelling the processes underlying these links; and agronomy, which assesses the effect of this diversity on broader spatial or temporal scales (rotation, farm, production basin). Each of these disciplines is distinguished by the scale at which it addresses the notion of diversity: the plant, the plot, the farm.

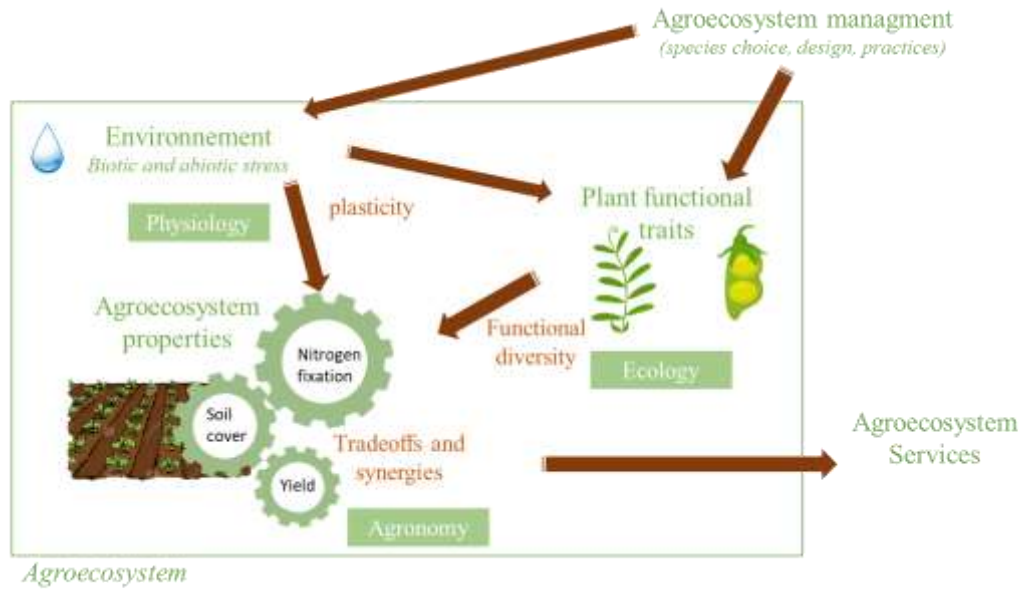


Figure 1 : an integrative approach based on functional traits and combining three disciplines : functional ecology, crop physiology, agronomy

We assume that plant functional traits is a notion that makes sense within these three disciplines and will be key to elaborate the required links between the different levels of investigation. Then we develop a trait-based approach to identify possible strategies by which multiple properties of agroecosystems can be jointly controlled for better provision of ecosystem services, which is a crucial aspect of agro-ecosystem management.

To answer the above questions and test related hypotheses, this thesis is arranged in three complementary sections through: (1) a meta-analysis of the functional diversity as collected in a literature survey, (2) an original study measuring functional diversity under agronomic conditions in comparable conditions, allowing a more precise analysis of traits and properties trade-offs, and (3) an analysis of the response of processes underlying some agro-ecosystem properties to water deficit.

- (1) Evaluation of pulse crops' functional diversity supporting food production (Guiguitant et al. 2020; article published in Scientific Reports)

We have collected several functional traits (*sensu* Violle *et al.*, 2007) in the literature, in the field and under controlled conditions to describe the functional diversity of pulses species. We selected whole-plant or plant organ characteristics, which we assumed to be good indicators of three ecosystem services supporting food production in a low-input system: 1) Yield under dry conditions; 2) Nitrogen fixation; and 3) Weed competitiveness.

The literature survey allowed us to synthesize and structure current knowledge on the functional diversity of legume crops at the interspecific level. The aim of this study was to provide a mapping of the functional space in which the groups of grain legumes is located and to highlight the (possible)

relationships with the provision of ecosystem services as well as possible trade-offs between these services. As ecosystem services are still poorly documented and poorly described in the existing literature, we focused on six properties of the agro-ecosystem that were assumed to be good indicators of the targeted services. Our comprehensive literature survey collected values for these six agro-ecosystem properties and a total of 17 treatments for 43 legume species.

- (2) Functional diversity of pulses' species and agro-ecosystem properties (article under review in *Journal of Applied Ecology*)

For this second section, we developed and monitored a large field experiment that allowed to acquire an original dataset complementary to the first database extracted from the literature. This experiment concerned 30 pulse varieties, belonging to ten species monitored in similar conditions. In accordance with the first study based on literature data (Guiguitant *et al.*, 2020), agro-ecosystem properties are defined in this study as traits or processes measured or calculated at the canopy level (Garnier *et al.*, 2007), such as biomass or the leaf area index. Traits of aboveground plant organs related to light-acquisition and nitrogen-nutrition were investigated. Measured agro-ecosystem properties gave insight on production services together with additional supporting services; namely, soil cover capacity and ability to use atmospheric nitrogen. This study also aimed at deciphering the impact of trait plasticity on predictions of agro-ecosystem properties. To do so, analyses were duplicated and compared when using experimental data collected within one uniform environment and literature data collected in diverse agronomic situations.

- (3) An exploration of the variability of physiological responses to soil drying in relation with C/N balance across the underutilized genus *Vigna* (Guiguitant *et al.* in press; article accepted in *Physiologia Plantarum*)

In order to investigate the functioning and variability of the responses of legumes subjected to water deficiency, an experiment in controlled conditions was carried out during a 4-month stay in the laboratory of Prof. Thomas Sinclair at North Carolina State University (NCSU). This study explores the physiological mechanisms of response to soil drying (N_2 fixation, transpiration rate and changes in C and N allocation) across three species of the *Vigna* genus: *Vigna radiata*, *Vigna unguiculata*, *Vigna vexillata* (tuber cowpea), as well as *Cicer arietinum*. Through the analysis of leaf traits variation (SLA, LNC) under well-watered and water-deficit conditions, we try to relate the variability in N_2 fixation and transpiration response to C/N metabolism modifications resulting in different allocation of carbon and nitrogen to leaves under water deficit.

D. References

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**CHAPTER 2. *EVALUATION OF PULSE CROPS'*
FUNCTIONAL DIVERSITY SUPPORTING FOOD
*PRODUCTION***

OPEN **Evaluation of pulse crops'
functional diversity supporting food
production**

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Abstract:

Pulses, defined as legumes which produce dry seed used for human consumption, are plants of great agronomic value, at the food system level as much as the field level but their diversity has been largely underused. This study aimed at analyzing existing data on cultivated grain legume species in the literature to provide a broad and structured description of pulses' interspecific functional diversity. We used a functional trait-based approach to evaluate how pulse diversity could support food production in agro-ecosystems constrained by low water and nutrient availability and exposed to high weed pressure. We gathered data for 17 functional traits and six agro-ecosystem properties for 43 pulse species. Our analytical framework highlights the correlations and combinations of functional traits that best predict values of six agro-ecosystem properties defined as ecosystem services estimates. We show that pulse diversity has been structured both by breeding and by an environmental gradient. The covariance space corresponding to agro-ecosystem properties was structured by three properties: producers, competitors, stress-tolerant species. The distribution of crop species in this functional space reflected ecological adaptive strategies described in wild species, where the size-related axis of variation is separated from variation of leaf morpho-physiological traits. Six agro-ecosystem properties were predicted by different combinations of traits. However, we identified ubiquitous plant traits such as leaflet length, days to maturity, seed weight, and leaf nitrogen content, that discriminated agro-ecosystem properties and allowed us to gather individual species into three clusters, representative of the three strategies highlighted earlier. Implications for pulses provisioning of services in agro-ecosystems are discussed.

A. Introduction

Over the past decade, grain legumes used for human consumption – pulses – have been receiving a resurgence of interest to meet agricultural challenges all over the world. Indeed, they are particularly important in human nutrition as sources of proteins, vitamins and minerals that complement a predominantly cereal-based diet (Singh *et al.*, 2003; Tharanathan and Mahadevamma, 2003). At field level, their well-known ability to fix atmospheric nitrogen helps reducing energy consumption while making them particularly suitable for low-input systems. They are also a source of diversification in order to break disease, pest and weed cycles and optimize nutrient management in standard crop rotations (Stagnari *et al.*, 2017). Although site-specific environmental constraints may reduce cropping systems options, the diversity of pulse, when fully explored, may offer solutions to most cropping systems, from a biophysical point of view. Indeed, leguminous plants constitute the third largest family among flowering plants and contain no less than 18,000 species (Edwards, 2007). This diversity implies potentially highly variable responses to abiotic and biotic stresses (Doyle, 2003). However, the specific diversity of pulse is poorly represented in most cropping systems despite the growing demand from food-chains (De Boer and Aiking, 2018). Most of pulse species could be defined as ‘neglected and underutilized species’ (NUS)(Cuevas, 1992; Foyer *et al.*, 2016a), i.e. useful plant species which are marginalized by researchers, breeders and policy makers (Padulosi, Thompson and Rudebjer, 2013). While more than 80 pulse species contribute to the human diet, the FAO database includes only 11 of these (Tiwari, Gowen and McKenna, 2011a). They are, most of the time, locally important crops maintained by cultural preferences and traditional practices because they are nutritionally rich (Padulosi, Eyzaguirre and Hodgkin, 1999; Ghane *et al.*, 2010) and adapted to harsh environments unfit for other crops where they can still provide sustainable productions (Mal, 2007). However, local perceptions often differ from global priorities, mostly for economic reasons, as a result NUS such as most legumes species, are not included in large breeding efforts, dissemination programs, or foresight studies. This lack of attention creates a risk of continuous erosion of expertise and genetic resources that could lead to a further limitation of development options aimed to reduce the perceived riskiness of such crops (Dansi *et al.*, 2012a). Limitations to a broader cultivation and use of pulses could be attributed to their weak recognition in food system and markets' bottom line (Magrini *et al.*, 2016). However, as recent articles (Massawe, Mayes and Cheng, 2016) demonstrate, diversity is a key component of a sustainable diet (Healthy Diet for Healthy Planet (Drewnowski, 2014)).

Beyond food security, the diversification of cropping systems with legumes could globally improve agro-ecosystem functioning (Ebert, 2014). Ecological research has demonstrated that ecosystem processes are determined by specific diversity. Increasing plant species richness may have a greater impact on ecosystem functioning at low diversity levels (Tilman *et al.*, 2001). This suggests that, in cropping systems composed of a few species, small increases in biodiversity using legume crops can

have a significant effect on ecosystem processes such as productivity or nutrient cycling (Jackson, Pascual and Hodgkin, 2007). Effects of biodiversity on agro-ecosystem properties can be evaluated through taxonomic diversity measures or, more recently, through plant functional traits (E Garnier and Navas, 2012a; Martin and Isaac, 2015b; Wood *et al.*, 2015b). The latter approach has the advantage of taking into account the significant differences between species, unlike "plant functional types" (PFTs), which often considers legumes as a homogeneous functional group (Lavorel and Garnier, 2002b; Martin and Isaac, 2015b). In light of these considerations, it is essential to help maintain and use pulse genetic resources, to ensure sustainable development and use by present and future generations, in order to take full advantage of the services they offer.

This study aims at synthesizing and structuring current knowledge on the diversity of cultivated pulses at the interspecific level. Rather than offering practical solutions to farmers, the purpose of this study is to provide a base map of the functional trait space of pulses and its (possible) relationship with individual or combined ecosystem service provision. We choose to do this evaluation through the plant functional traits approach as defined earlier (E Garnier and Navas, 2012a; Martin and Isaac, 2015b; Wood *et al.*, 2015b). We assume that this approach could help to identify possible strategies by which multiple agro-ecosystem properties can be jointly controlled for a better ecosystem services provision, which is a crucial aspect of agro-ecosystem management. Here we define agro-ecosystem properties as traits or processes measured or calculated at the crop level (Garnier *et al.*, 2007), such as biomass or the leaf area index. To this end, we gathered several functional traits (Violle *et al.*, 2007) in the literature to describe pulse species. Selected traits were characteristics of an individual plant or plant organ, which can be morphological, physiological or phenological and that are supposed to have direct or indirect effects on agro-ecosystem properties. Since ecosystem services are still poorly described, we focused on six agro-ecosystem properties hypothesized to be good indicators of three specific ecosystem services related to food provision in a low-input systems: 1) yield under dry conditions; 2) nitrogen fixation; and 3) competitiveness toward weeds. We discuss the potential of this approach to orientate breeding efforts and elicit conservation programs when further site specific and agro-economic evaluations, that are essential to support actual integration of pulse diversity into farmer's fields, would have been performed.

B. Material and methods

I. Approach

Our extensive literature review allowed to collect values for these six agro-ecosystem properties as well as a total of 17 traits for 43 pulses species. We discuss the combinations of pulses' functional traits that may favor individual or combined ecosystem services provision.

II. Data sources

Table 1 : Functional traits collected for 43 species of pulses.

Trait name	abbreviations	Unit	% of missing value
Days to maturity	DM	Days	0 %
Days to flowering	DF	Days	0 %
Seed diameter	Seed_size	mm	0 %
Number of leaflet	lft_nb	number	0 %
Leaflet width	Lft_wth	mm	2.3 %
Leaflet length	Lft_lth	mm	2.3 %
Plant height	PH	cm	0 %
Habit		Erect, climbing	0 %
Germination type		Epigeal, hypogeal	0 %
Leaf nitrogen content	LNC	mg g ⁻¹	9.3 %
Thousand seed weight	TSW	g	0 %
Grain protein content	Seed_prot	%	0 %
Grain oil content	Seed_oil	%	0 %
Leaf area per plant	LA	cm ²	18.6 %
Specific leaf area	SLA	cm ² g ⁻¹	16.3 %
Shoot to root ratio	S/R	-	9.3 %
Harvest index	HI	-	23.2 %

We compiled a database from 327 published studies where at least one plant trait or agro-ecosystem property was measured on a pulse species (see complete reference list in Annexe 1). We selected 17 functional traits measured at the sub-individual or individual level, based on data completeness (Table 1). We were able to document 43 pulse species on most of the traits selected (~5% of missing values) (Table 1). The complete list of covered species is provided in supplementary materials (Supplementary Table S1). Species covered the two clads that gather the majority of legume crops, namely Galegoid and Phaseoloid, which are often referred to as cool season and tropical season legumes, respectively (Doyle, 2003).

We also gathered data on six agro-ecosystem properties measured at the supra-individual (population) level, but these data were less complete than the traits (~17% missing values) (Table 2). These six properties have identified relations with targeted ecosystem services.

Table 2 : Agro-ecosystem properties collected for 43 species of pulses.

Agro-ecosystem function (services indicator)	Abbreviation	unit	% of missing data
Grain yield	GY	t.ha ⁻¹	2.3 %
Biomass yield	BY	t.ha ⁻¹	7 %
Water use efficiency	WUE	kg.ha ⁻¹ .mm	25.6 %
Percentage of nitrogen derived from atmosphere	%Ndfa	%	18.6 %
Yield loss (due to weeds)	%YR	%	30.2 %
LAI	LAI	-	16.3 %

We considered that food provision under drought is a function of grain yield and water use efficiency (WUE) (Equation 1).

$$Food\ services_{drought} \leftarrow \frac{Yield}{Tr} \times water = WUE \times water \quad (Equation\ 1)$$

Nitrogen supply service is predicted to be tightly related to biological nitrogen fixation (BNF) which is a function of the percentage of nitrogen derived from atmosphere (%Ndfa) and biomass yield (BY) (Equation 2).

$$Nitrogen\ supply \leftarrow BNF = biomass \times \%Ndfa \times [N]total \quad (Equation\ 2)$$

Finally, competition against weeds was assessed by grain yield loss when the crop is subject to competition which we supposed to be function of maximum crop leaf area index (LAI) divided by the time needed to reach the maximum LAI (Equation 3).

$$Weed\ interference \leftarrow \Delta Yield \leftarrow f\left(\frac{LAI_{max}}{Flowering-Germination}\right) \quad (Equation\ 3)$$

Data originated from very diverse sources (scientific literature, flora, crop guides, databases) since no study have reported data for all the traits for one species. For a given species, values for a given trait or a given agro-ecosystem property may differ depending on the genotype (G), environment (E) or cropping practice (M) that was applied to crop. Although GxExM effects add more variability to the dataset, it was impossible to separate it from the interspecific effect as no published literature would compare species as diverse as tropical perennial pulse species versus cool season short cycle pulse crops. To limit over-weighting of extreme values and sampling effect, we collected more than one value per trait and species and average was used as an aggregation function. Moreover, it has been demonstrated that species trait values are consistent enough to allow values to be used from different data sets (experiments, databases) to characterize local populations of species (Kazakou *et al.*, 2014).

III. Data analysis

We initially focused on the linkages between agro-ecosystem properties, in order to get an overview of the potential trade-offs between services. A principal component analysis (PCA) was conducted on a reduced number of species ($n = 22$) still representative of the two clades that gather the majority of legume crops.

In order to describe the functional diversity of pulse, we performed a probabilistic principal component analysis (PPCA) on functional traits. This is a dimensionality reduction technique that analyzes data via a lower dimensional latent space (Tipping and Bishop, 1999). It is often used when there are relatively low levels of missing values in the data or for multidimensional scaling.

To identify the best explanatory pulse functional traits for predicting agro-ecosystem functioning, we performed a classification and regression tree (CART) analysis. These prediction models are obtained using machine-learning algorithms that recursively partition the data space in order to fit the simplest prediction model within each partition. The resulting partitioning can be represented graphically as a decision tree (Breiman *et al.*, 1994). Six trees were built to predict each agro-ecosystem property with trait values extracted from the PPCA. To control the effect of having only few trait values for some species and of GxExM interaction, we conducted a sensitivity analysis on the effect of trait value sampling on the results of the CART analysis. A hierarchical clustering was performed on the 43 species based on the traits revealed by CART regression as the best predictors of agro-ecosystem properties.

All statistical analyses were performed in the computing environment R 3.5.2 (R Core Team, 2018) using *pcaMethods* package (Stacklies *et al.*, 2007) and *rpart* package (Therneau *et al.*, 2015).

C. Results

I. Variability of pulse crops agro-ecosystem properties

The first three principal components (PC) altogether explained 74% of the variability of agro-ecosystem properties with the first PC (33%) strongly positively associated with biomass yield (BY) and grain yield (GY) and to a lesser extent %Ndfa (percentage of nitrogen derived from atmosphere) (Figure 2a). This axis opposed high yield producers like *Canavalia ensiformis* (GY $2.53 \pm 2.8 \text{ t ha}^{-1}$, BY $9.79 \pm 2.29 \text{ t ha}^{-1}$) to poor yield providers like *Cymopsis tetragonoloba* (GY $1.01 \pm 1.3 \text{ t ha}^{-1}$, BY $1.42 \pm 0.5 \text{ t ha}^{-1}$), respectively representative of the phaseolids clade (warm season legume) and sister the indigoferoid clade.

The second PC axis (24%) opposed LAI (leaf area index) to percentage of yield reduction due to weeds (%YR) and GY (Figure 2a), but the correlations between the second PC axis and respectively, LAI and %YR were both low ($r = -0.15$, $P = 0.5$; Supplementary Table S2). %YR was also found to be only slightly correlated to GY ($r = 0.27$, $P = 0.22$). This could suggest that LAI alone is not enough to predict species ability to produce high yield under strong weed competition. The second axis opposed *Lathyrus ochrus* (LAI 4.1 ± 0.3 , %YR 5%) and *Lens culinaris* (LAI 3.9 ± 2 , %YR 2 ± 1 %), both representative of Galegoid clade, to *Cyamopsis tetragonoloba* (LAI 1.4, %YR 79 ± 6 %) and *Glycine max* (LAI 2.7 ± 1 , %YR 60 %), representative of the phaseolids clade.

The third axis explained 16% of the variability and opposed WUE (water use efficiency) and %Ndfa (Figure 2b). As expected, GY and BY were significantly correlated ($r = 0.69$, $P < 0.001$; Supplementary table S 2). PCA results associated %Ndfa to yield on the first axis, yet it was only slightly correlated to BY (Figure 2; $r = 0.22$; Supplementary Table S2). This property (%Ndfa) along with WUE is well represented on axis 3 which opposed (Figure 2c) *Vigna unguiculata* (%Ndfa 24.7 ± 0.5 %, WUE 8.3 ± 10), representative of phaseolids clade, to *Lathyrus sativus* (%Ndfa 93.3 ± 3.5 %, WUE 6.3 ± 3.1), representative of Galegoid clade. Pearson's correlation coefficient showed a very slight positive, but not significant, correlation of WUE with LAI ($r = 0.35$, $P = 0.11$). Quality of the representation of WUE in the PCA was low; the property was slightly associated with the three axes with low explain of variation explain on each axes, and it was also slightly but not significantly correlated with BY ($r = 0.27$, $P = 0.22$).

The projection of individual species in the space of covariation showed that species characterized by high LAI and good tolerance to weed infestation (*Lathyrus ochrus*, *Lens culinaris*, *Lathyrus sativus*, *Vicia sativa*), are relatively unproductive species with GY varying from 0.6 to 1.1 t ha^{-1} and BY from 2.3 to 3.4 t ha^{-1} . Moreover, they seemed relatively adapted to nitrogen deficiency and drought. Except for *Lens culinaris*, which showed a low nitrogen fixation efficiency, %Ndfa varied from 69% to 93%, while WUE varied from 6.05 to $7.5 \text{ kg ha}^{-1} \text{ mm}^{-1}$. However, relatively unproductive species could be

sensitive to weed infestation, especially as they show a lower WUE (*Vigna radiata*, *Vigna mungo*) or a lower %Ndfa (*Vigna unguiculata*).

Productive species (GY from 1.1 to 2.5 t ha⁻¹ and BY from 3.9 to 10.6 t ha⁻¹) can be sensitive or insensitive to weed infestations (28 to 83 % of yield reduction), and this ability cannot be related to nitrogen or water adaptations. However, productive species, except for *Phaseolus lunatus*, show a high value of WUE (from 5.2 to 10.4 kg ha⁻¹ mm⁻¹) and %Ndfa (from 61 to 82%).

Cyamopsis tetragonoloba is found apart from every other species in the space of covariation as it showed low values on every agro-ecosystem property.

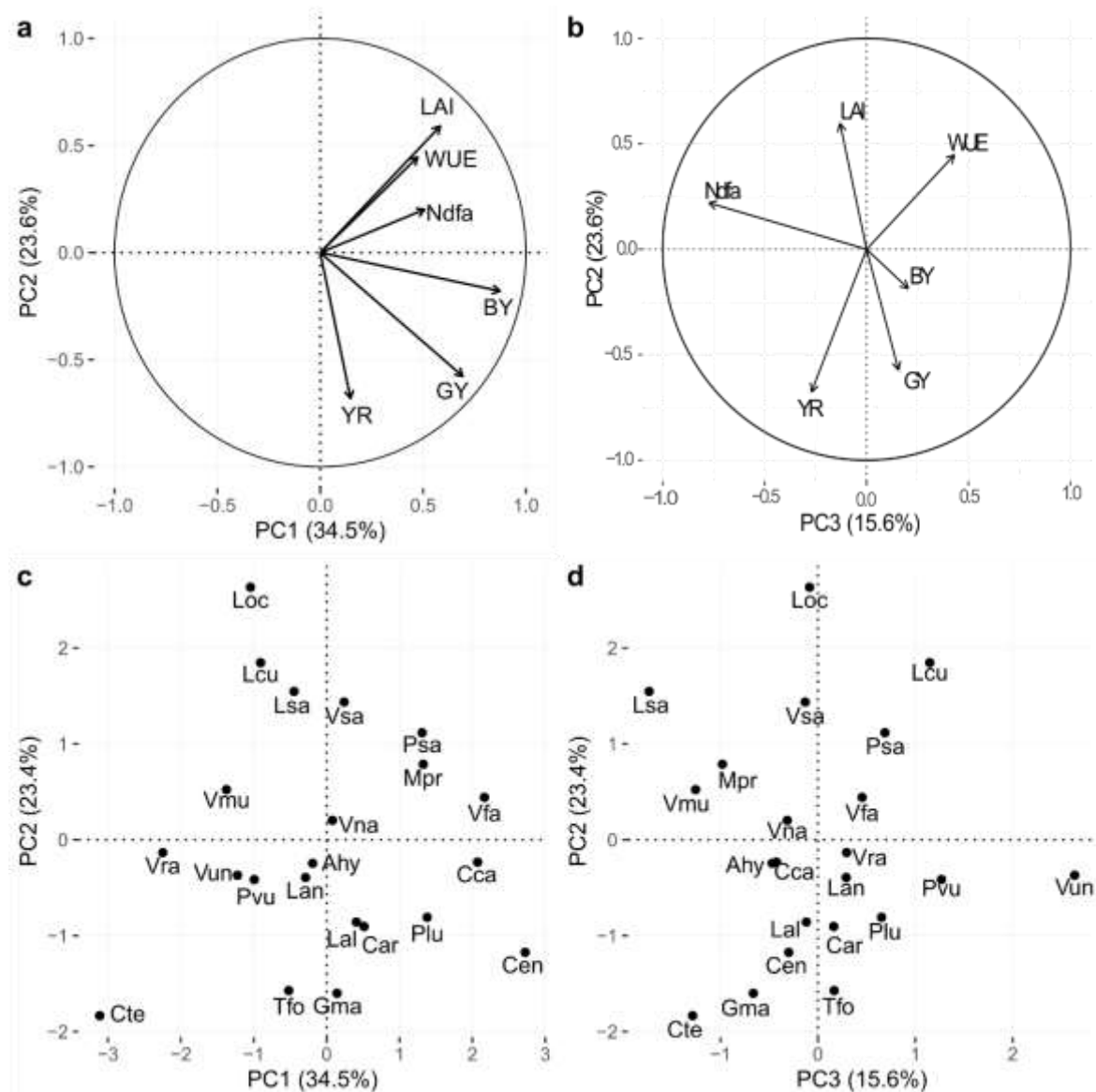


Figure 2 : Principal component analysis (PCA) performed on six agro-ecosystem properties collected for 22 species of pulses. Visualization of the variables and correlation circles on (a) PC1-PC2 and (b) PC2-PC3 planes. (c, d) Individual species projection on the first three axes of the PCA. See Table 2 for abbreviations of agro-ecosystems properties and Supplementary Table S1 for abbreviations of species.

II. (Co-)variations of functional traits

The first three PC axes of the PPCA (probabilistic principal component analysis) performed on functional traits collected for the 43 species explained 61% of total variance (Figure 3).

PC1 (33%) was associated with morphological traits, especially leaf traits (e.g. leaflet length, leaflet width, leaflet number, leaf area, plant height, and to a lesser extend seed diameter and seed weight). This axis opposed small plants such as *Astragalus sp.* (i.e. *A. hamosus*: seed diameter 2.1 ± 0.4 mm; TSW 17.7 ± 18.9 g; 21 ± 4.2 leaflets; leaflet length 9.8 ± 1.0 mm; plant height 55.9 ± 4.1 cm, LA 48.3 ± 19.6 cm²) to large plants such as *Canavalia sp.* (i.e. *C. gladiata*: seed diameter 23.3 ± 10.4 mm; TSW 1007.5 ± 382.3 g; tri-foliolate; leaflet length 137.5 ± 72.2 mm; plant height 432.9 ± 186.8 cm; LA 143.7 ± 15.9 cm²). The number of leaflets is characteristic of the species' origin; more than half ($n = 26$) of the species are trifoliolate, most of them being tropical legumes except for a small number of Mediterranean species ($n = 5$) with a number of leaflets ranging from 1 to 4 leaflets per leaf. The remaining ($n = 17$) have many leaflets, ranging from 5 to 24; this was the case of Mediterranean and European species only. Number of leaflets showed significant negative correlation to leaflet length ($r = -0.81$, $P < 0.001$; Supplementary Table S3) which ranged from 9.8 ± 1.0 mm (*Astragalus hamosus*) to 137.5 ± 72.2 mm (*Canavalia gladiata*). Plant height (PH) ranged from 11.3 cm (*Cicer reticulatum*) to 432.9 ± 186.8 (*Canavalia gladiata*) and averaged 103.1 ± 91.8 cm. LA ranged from 21.11 cm² (*Vicia ervilia*) to 6885.4 ± 5324.5 cm² (*Psophocarpus tetragonolobus*) and was positively correlated to all leaflet traits and PH; leaflet number ($r = -0.56$, $P < 0.001$), leaflet length ($r = 0.61$, $P < 0.001$), leaflet width ($r = 0.55$, $P < 0.01$) and PH ($r = 0.44$, $P < 0.01$; Supplementary Table S3).

PC2 (15%) was strongly associated with seed traits such as thousand seed weight (TSW), seed diameter and seed oil and protein content (Figure 3a). This axis opposed species with small, low quality seeds (i.e. *Vigna radiata* oil content 0.97 ± 0.2 %; protein content 25.2 ± 1.8 %; seed diameter 2.75 ± 1.8 mm; TSW 39.9 ± 11.1 g) to species producing larger seeds with higher oil and protein content (i.e. *Lupinus mutabilis* oil content 18.2 ± 5.2 %; protein content 45.4 %; seed diameter 11 ± 4.5 mm; TSW 286.75 ± 171.2 g) (Figure 3c). Seed diameter ranged from 2.1 ± 0.3 mm (*A. annularis*) to 23.3 ± 10.4 mm (*C. gladiata*) with a mean of 7.8 ± 4.5 mm. TSW was closely correlated with seed diameter ($r = 0.87$, $P < 0.001$; Supplementary Table S3) and ranged from 12.8 ± 3.9 g (*Trigonella foenum-graecum*) to 1178 g (*Phaseolus coccineus*) and averaged 255.4 ± 294.1 g. Seed protein content ranged from 10.9 % (*Canavalia ensiformis*) to 45.4 % (*Lupinus mutabilis*) and was moderately correlated to seed oil content ($r = 0.45$, $P < 0.01$; Supplementary Table S3) which varied greatly from 0.4 % (*Vigna angularis*) to 47.1 ± 1.9 % (*Arachis hypogaea*) but with a dominance of low values (mean: 4.8 ± 8.4 %).

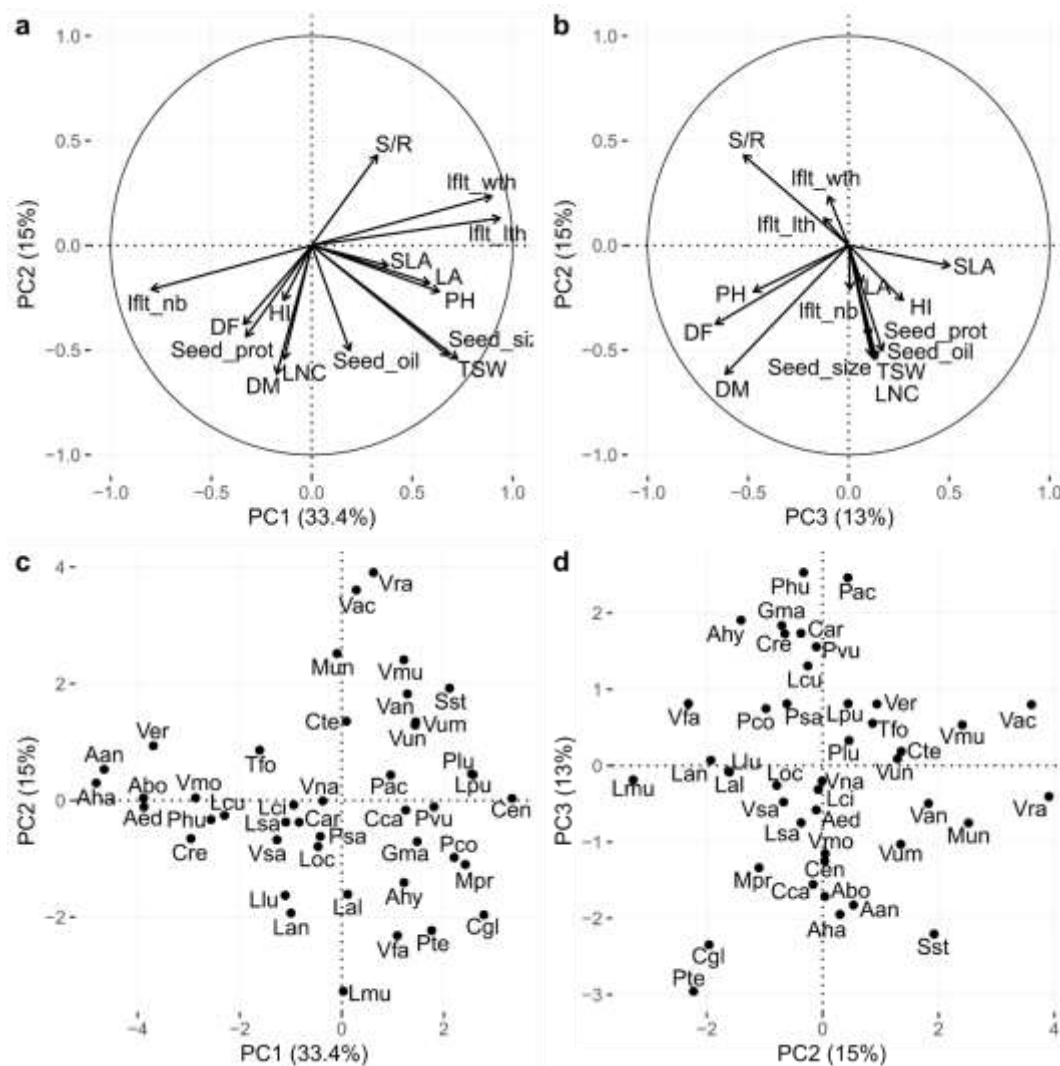


Figure 3 : Probabilistic PCA performed on 15 functional traits collected for 43 pulses species. Visualization of the variables and correlation circles on (a) PC1-PC2 and (b) PC2-PC3 planes. Individual species projection on the first three axes of the PCA (c, d). See Table 2 and Supplementary Table S1 for abbreviations of agro-ecosystems properties and Supplementary Table S1 for abbreviations of species.

The third PC axis (13.7%) opposed shoot/root ratio (S/R), days to maturity (DM), days to flowering (DF) and PH to specific leaf area (SLA) and leaf nitrogen content (LNC) (Figure 3b). It opposed species with fast strategies for nutrient acquisition and use (high LNC and SLA) as *Phaseolus acutifolius* (DM 85 ± 26 days, DF 33 ± 9 days, LNC 43.5 mg g^{-1} , SLA $420 \text{ cm}^2 \text{ g}^{-1}$) to species with slower strategies mostly based on their reproductive timing (DM and DF) such as *Astragalus boeticus* (DM 177 ± 3 days, DF 115 ± 5 days, LNC $19.3 \pm 13.1 \text{ mg g}^{-1}$, SLA $100 \pm 25.3 \text{ cm}^2 \text{ g}^{-1}$) or *Canavalia gladiata* (DM 202 ± 9 days, DF 122 ± 9 days, LNC 23 mg g^{-1} , SLA $253. \text{ cm}^2 \text{ g}^{-1}$). S/R ratio ranged from 1.2 ± 1.2 (*Phaseolus acutifolius*) to 19.4 ± 2.1 (*Sphenostylis stenocarpa*), its coordinate on the third axis was positive but S/R was not significantly correlated to DM and DF. Yet, it was negatively correlated to LNC ($r = -0.4$, $P < 0.01$) and positively to leaflet width ($r = 0.52$, $P < 0.001$). DM ranged from 79.5 ± 29.8 days (*Vigna radiata*) to 255 ± 148.5 days (*Lupinus mutabilis*) and was strongly correlated with DF ($r = 0.69$, $P < 0.001$) which ranged from 30 ± 35 days (*Cyamopsis tetragonoloba*)

to 139.25 ± 30.6 days (*Astragalus hamosus*). SLA ranged from 39.2 ± 33.2 cm² g⁻¹ (*Psophocarpus tetragonolobus*) to 537.2 ± 97.3 (*Lablab purpureus*). LNC ranged from 19.3 ± 13.1 (*Astragalus sp.*) to 60.2 ± 2.4 (*Lathyrus sativus*).

III. Relationships between functional traits and agro-ecosystem properties

CART analysis showed that grain legume species with SLA above 274 cm² g⁻¹ (average 245.5 ± 105.4) produce more biomass (Figure 4). BY is maximal (8 t ha⁻¹) for high SLA combined with TSW above 37 g (which concerns most of the species as only nine species have smaller seeds), and PH above 83 cm (not far from the average 103.1 ± 91.8). BY around 4-5 t ha⁻¹ can be achieved by small species (< 83cm) with high SLA, and doesn't seem to be related to seed weight. Low SLA species can still achieve intermediate BY if they have small seeds. Among the lowest biomass producers (small SLA, big seeds) erect species and tall species have higher BY.

GY is maximal (2.2 t ha⁻¹) for species which take more than 168 days to mature (average 135.0 ± 38.4) (Figure 5). In early maturing species, GY is maximized when LNC is higher than 40 mg g⁻¹ (average 38.4 ± 12.1) in species that produce big seeds (TSW > 164 g). In small-seeded species (TSW < 164 g), there is no relation between LNC and GY whereas erect habit generally leads to higher GY than climbing habit. Rapid crop cycle combined with small seeds generally leads to low GY with one exception for tall species (PH ≥ 61 cm) with erect habit.

The number of days to maturity (DM) was also discriminant for %Ndfa. This agro-ecosystem property is indeed maximized (66-78%) for late maturing (DM ≥ 142 days) species, among which small-seeded species (TSW < 147 g) fix more nitrogen from the atmosphere than large-seeded species (TWS ≥ 147 g; Figure 6). A slightly smaller amount of nitrogen fixation could also be reached by early maturing species with smaller leaflets length (< 101 mm) and S/R specifically above 4.8. Yet, the advantage of a large S/R ratio was only observed in this situation, while early maturing species with long leaflets exhibit low %Ndfa.

In our data analyses, LAI of pulses is sequentially smaller in small-seeded species that have high protein content in their seeds, an epigeal germination and small leaflets (Figure 7).

Yield of species with very small leaflets (length < 30 mm) is less negatively impacted by weeds (Figure 8). However, leaflet length under 30 mm is only encountered in four species over the 25 and two of them are under 10% YR which is extremely low compared to other species. For the remaining 21 species, DM was again a crucial trait. Short cycle (< 103 days to maturity) leads to reduced yield losses due to weeds (under 60%). Yet, a long crop cycle duration combined with hypogeal germination and a better harvest index (HI) than average (≥ 0.29) also resulted in a similar percentage of yield loss due to weeds (43%). Among early maturing species, yield of those that have the highest oil content in seeds is less affected by the presence of weeds.

WUE ($8.3 \text{ kg ha}^{-1} \text{ mm}^{-1}$) is maximized in species with high LNC ($\geq 50 \text{ mg g}^{-1}$) (Figure 9). While in species with low LNC those with small leaves have the lowest WUE, it is noticeable that in species with larger leaves could have a low WUE is significantly higher for those that have the lowest seed oil content.

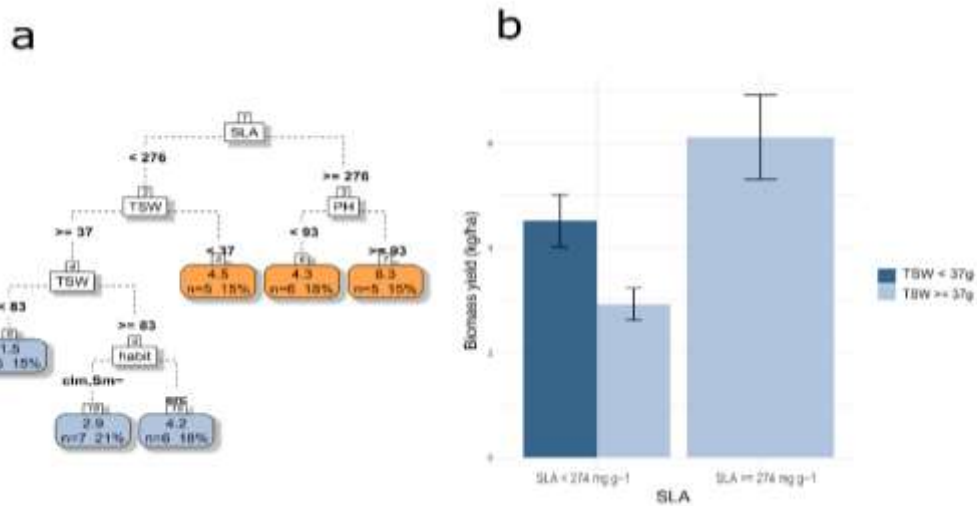


Figure 4 : (a) CART regression tree for prediction of biomass yield of pulses species ($n = 37$). Root nodes represent single input variables (functional traits) and related split point used to make the prediction. Leaf nodes contain mean, number and percentage of observations of the predicted variable. (b) Mean and standard error of nodes 2 and 3. See Tables 1 for abbreviations

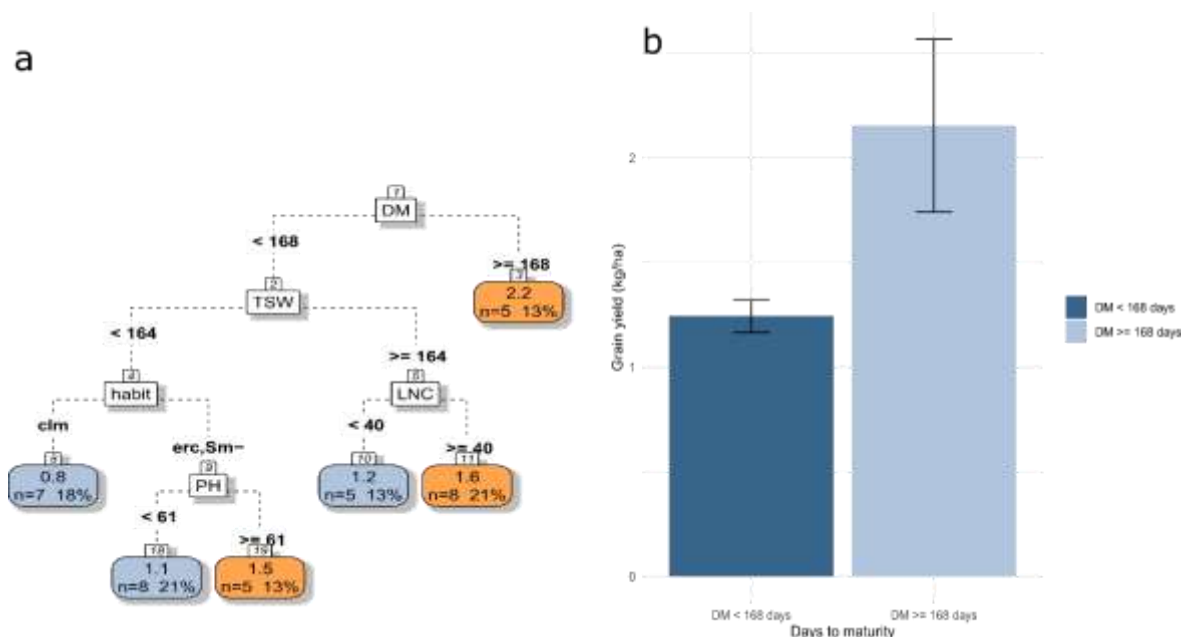


Figure 5 : (a) CART regression for prediction of grain yield of pulses species ($n = 39$). Root nodes represent single input variables (functional traits) and related split point used to make the prediction. Leaf nodes contain mean, number and percentage of observations of the predicted variable. (b) Mean and standard error of nodes 2 and 3. See Table 1 for abbreviations.

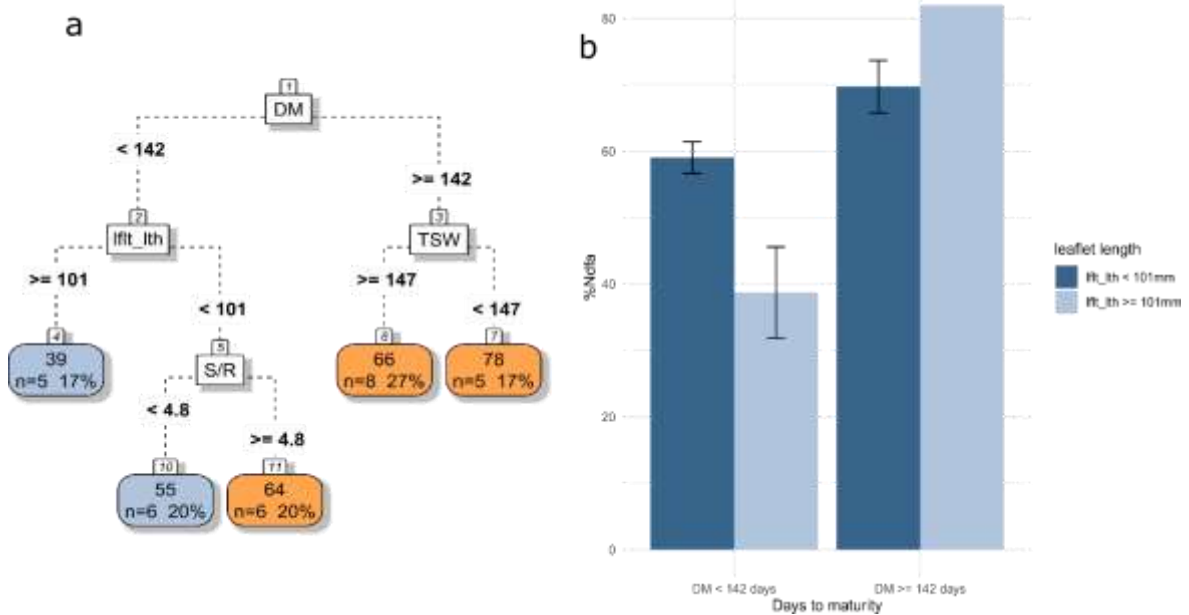


Figure 6 : (a) CART regression for prediction of %Ndfa of pulses species (n=30). Root nodes represent single input variables (functional traits) and related split point used to make the prediction. Leaf nodes contain mean, number and percentage of observations of the predicted variable. (b) Mean and standard error of nodes 3, 4 and 5. See Table 1 and 2 for abbreviations.

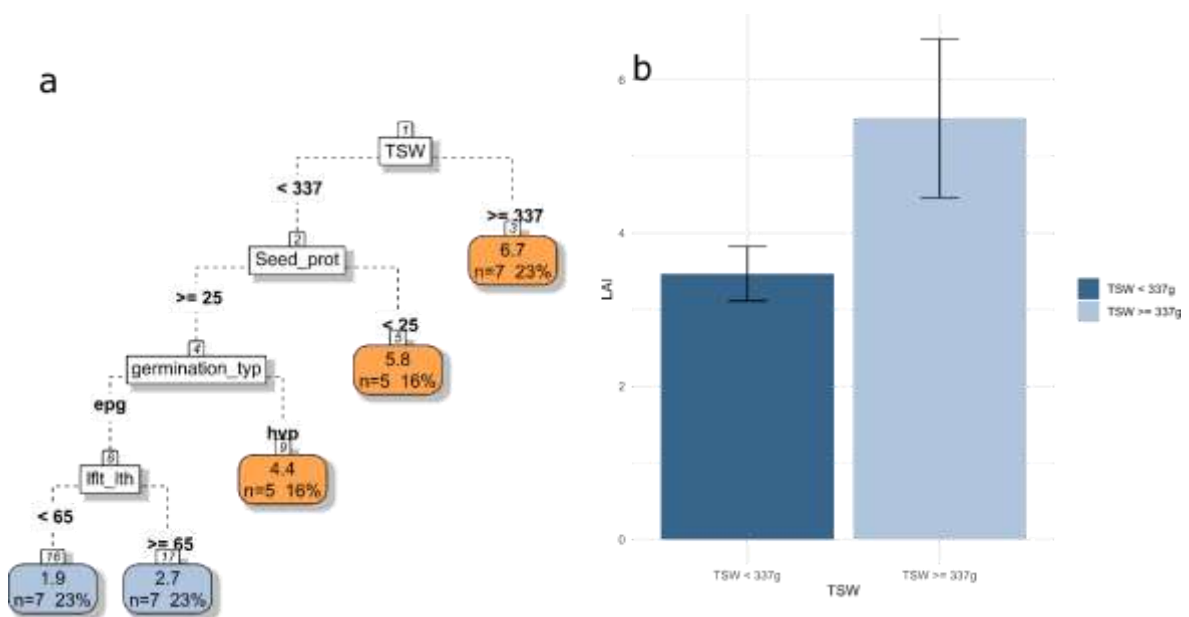


Figure 7 : (a) CART regression for prediction of LAI of pulses species (n=31). Root nodes represent single input variables (functional traits) and related split point used to make the prediction. Leaf nodes contain mean, number and percentage of observations of the predicted variable. (b) Mean and standard error of nodes 2 and 3. See Table 1 and 2 for abbreviations.

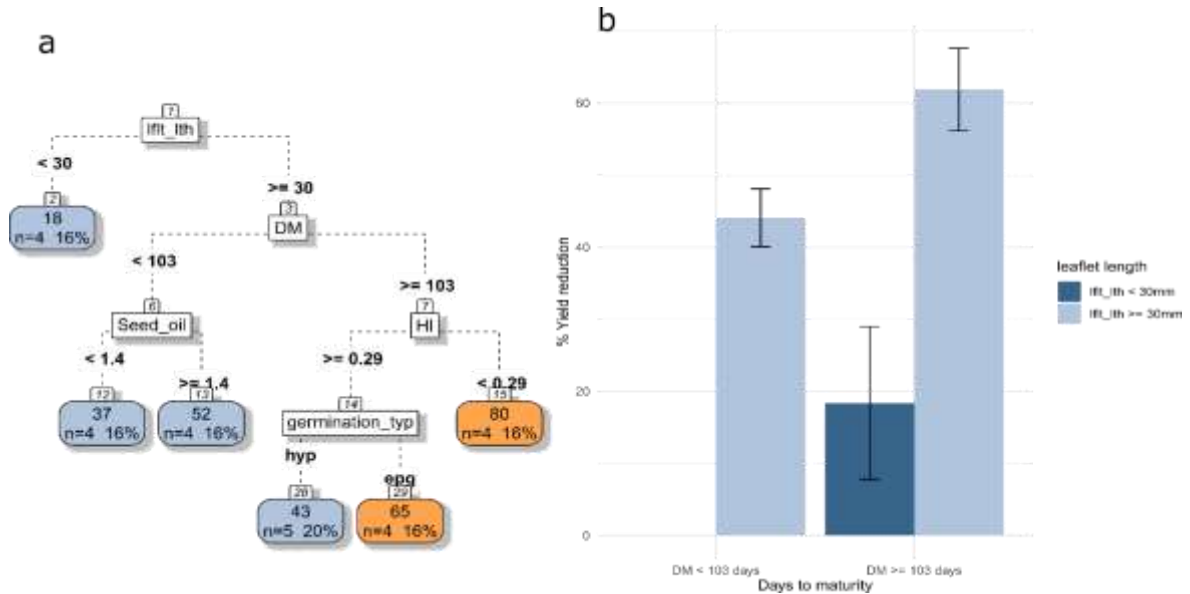


Figure 8 : (a) CART regression for prediction of yield reduction of pulses species ($n=25$). Root nodes represent single input variables (functional traits) and related split point used to make the prediction. Leaf nodes contain mean, number and percentage of observations of the predicted variable. (b) Mean and standard error of nodes 2, 6 and 7. See Table 1 and 2 for abbreviations

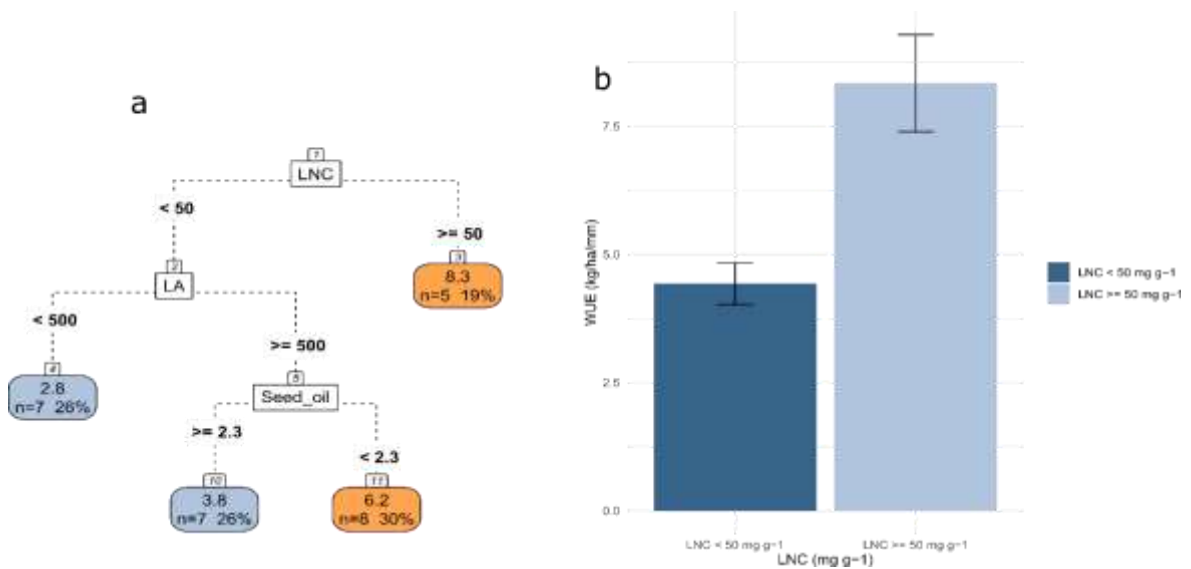


Figure 9 : (a) CART regression for prediction of WUE of pulses species ($n=28$). Root nodes represent single input variables (functional traits) and related split point used to make the prediction. Leaf nodes contain mean, number and percentage of observations of the predicted variable. (b) Mean and standard error of the node 2 and 3. See Table 1 and 2 for abbreviations.

We conducted a sensitivity analysis to control the effect of sampling on CART result. We considered 50 random samples containing 75 % of the trait values collected in the database and repeated the CART analysis for each agro-ecosystem property. Sensitivity analysis revealed that trait value sampling would not affect the CART results significantly for all functions except WUE. Indeed, in more than 50% of the regressions, the top three segregating traits were found to be the same as when considering the average of collected values for each trait per species (data not shown).

A hierarchical clustering analysis classified pulses in three groups (Supplementary figure S1) with a range of agro-ecosystem property values that were in accordance with PCA results, that is, high BY and GY for cluster 1, high %Ndfa and WUE for cluster 2 and low LAI and high %YR for cluster 3. Details about the classification of species based on the values of the five top traits for property prediction (SLA, LNC, TSW, DM, leaflet length), and species cluster values for each agro-ecosystem property are given in Supplementary Materials (Supplementary figure S1).

D. Discussion

In this study, we aimed at quantifying the pulse' interspecific functional diversity and its relationships with the provisioning of ecosystem services, therefore providing a broad base map for future design and evaluation of diversified cropping systems. Quantification of ecosystem services is most often achieved through monetary evaluation, which requires many hypotheses that are mostly context dependent (Cordier *et al.*, 2014). In addition, ecosystem services in agriculture are essentially those supporting or regulating production services, such as the three services we have targeted to support food production, by improving soil fertility, climatic limitation or pest regulation (Jordan *et al.*, 2011). Quantifying ecosystem services as if they were independent from each other can be in vain. Instead, we focused on agro-ecosystem properties which were available in the literature and hypothesized as indicators of ecosystem services. We followed a functional traits approach to draw comprehension of how crop traits could influence these properties and, as a result, the overall service production of an agro-ecosystem. This approach explicitly assumes that relationships exist between traits as well as between agro-ecosystem properties in order to better decipher the trade-offs between services and their causes of variation. We have selected agro-ecosystem properties that were related to crop yield, water use efficiency, nitrogen fixation and ability to maintain yield under competition with weeds.

I. Plant production strategies among pulse species

Collected values for the six agro-ecosystem properties revealed three main axes of variation. The first axis sorted pulses based on their biomass and grain production level. The second axis was mostly related to response to competition, especially against weeds, while the third axis sorted pulses based on their ability to fix nitrogen. Despite its wide variation among pulses, water use efficiency was poorly represented and occupied an intermediate position in this space of variation.

These results might highlight segregation between species that are adapted to productive versus unproductive or competitive environments. This is consistent with Grime's CSR triangle theory that predicts plant strategies are selected along environmental gradients of resource availability, stresses and perturbation (Grime *et al.*, 1997a; Grime, 2009a). Grime's theory highlighted that these gradients might have selected optimized trade-offs between phenology or development and resource acquisition in plants. This results in a CSR classification based on a combination of three extreme plant strategies: competitors (C), stress-tolerant (S), and ruderals (R) (Pierce *et al.*, 2017). When applied to Mediterranean pasture legumes such as *Medicago sp.* (Yousfi *et al.*, 2010), *Trifolium sp.* (Norman, Cocks and Galwey, 2002) or *Lupinus sp.* (Berger, Shrestha and Ludwig, 2017), this theory was in accordance with the variation of traits related to water acquisition and use, and highlighted contrasted plant adaptations to drought (Berger and Ludwig, 2014).

In the species set surveyed here, the third axis might partially represent species adaptation to stressful environments with low levels of nitrogen and water. The first axis of the PCA sorted the species on their productivity ability selected in potentially highly productive environments such as fertilized and irrigated crop systems associated with limited competition due to recurrent perturbations (mechanical or chemical destructions). This axis could be associated to R-strategies. However, productive species could be more or less sensitive to competition mostly depending on their ability to intercept light as suggested by the second axis of the PCA. This competitive ability might have been selected in highly productive and crowded environments and be related to C-strategy. However, agro-ecosystem are ruderal environments: pulses as crop species have a peculiar evolutionary history compared to uncultivated species due to their breeding background. They played an important role in human nutrition and thus might have been more often managed under non-limiting conditions (irrigation and fertilization) and would exhibit high grain production levels compared to species with less importance and that were predominantly used for feed. Thus, it is more likely that axes 1 and 2 show classification of competitive ability inside ruderal strategies.

Finally, species that were considered in this study came from a wide range of geographic origins and have therefore been subject to a great diversity of climates, farming practices and resources along their history, leading to contrasted adaptations to stressful conditions, where nitrogen fixation may be determining in competitive capacity. In the current context of climate change and reduction of resource availability, species that are able to maintain relatively high productivity under stressful conditions could be valuable for the provision of food.

11. Patterns of trait covariations and plant production strategies

Our results showed trait covariation patterns consistent with those predicted and observed by common Plant Ecological Strategies Schemes (PESS) previously described in wild species (Grime *et al.*, 1997a; Westoby, 1998; Saugier, 2001; Moles *et al.*, 2002; Lavergne, Garnier and Debussche,

2003; Quétier *et al.*, 2007). Leaf, seed or growth strategy are major components of CSR strategy variation. Unfortunately, we were unable to position pulse crops in the Grime's CSR triangle (e.g. using the computation (Pierce *et al.*, 2017) due to the lack of trait values.

We found that seed and leaf traits exhibited the greatest variability among the 43 pulse species, in accordance with Westoby's LHS (leaf-height-seed) scheme (Westoby, 1998). The first PC axis was best explained by leaf size, leaf area and, especially, plant height, which is recognized as a good indicator of plant capacity for competitive dominance (Gaudet and Keddy, 1988; Westoby, 1998; Hodgson *et al.*, 1999). Variation of seed size and TGW was mostly associated to the second axis of our PCA but had an intermediate position in the first PC plan and indicated that the seed dimension axis defined by Westoby (1998), as an expression of the potential of dispersal and successful establishment of a species (Cipollini and Stiles, 1991; Willson, 1993; Askew *et al.*, 1997), was marginally correlated to plant and leaf size dimensions in the studied pulses; this result was consistent with previous findings by Leishman (Leishman *et al.*, 1985). The third dimension of the LHS scheme relates to the capacity of plants to exploit resource-rich and resource-poor environments. This dimension reflects the trade-off between "fast" traits that promote rapid resource acquisition and "slow" traits that promote resource conservation (Wright *et al.*, 2004a; Díaz *et al.*, 2016; Volaire, 2018). In the search for a single trait that captures the core of this axis, specific leaf area (SLA) is a leading contender (Poorter and Remkes, 1990; Lambers and Poorter, 1992; Poorter *et al.*, 2009). However, our study shows that this trait contributed only marginally to the total trait variation among pulses through its contribution to the third PC axis. Part of pulse variability represented on the third PC axis was best represented by days to maturity and flowering, which can also be negatively related to plant capacity to rapidly acquire resources.

As previously observed by Tribouillois (Tribouillois, Cruz, *et al.*, 2015) for herbaceous Fabaceae crops, the relationship between SLA and LNC was similar to that observed across a large diversity of wild species (Wright *et al.*, 2004a; Díaz *et al.*, 2016) although LNC values of N-fixer species were on average higher than non N-fixer species (see Supplementary figure S2). The total percentage of variability explained by the first three PCs (61%) was relatively low compared to what is usually observed in wild and cultivated species (Wright *et al.*, 2004a; Díaz *et al.*, 2016). This could be attributed to the collection method of data and/or the peculiarities of these cultivated Fabaceae species.

III. From trait profiles to services

Functional traits are directly or indirectly linked to ecological processes leading to agro-ecosystem properties. Several traits can be involved in one process and one trait can participate in several processes. Thus, a given trait can only predict ecosystem properties value as long as every other trait have fixed values and processes occur at a fixed rate. CART regressions were particularly relevant

to overcome this issue. For example, our results show that high WUE efficiency could be achieved by plants with LNC over 50 mg g⁻¹ or, alternatively, with low LNC if total leaf area was above 500 cm². Similarly, late maturing species matched high yield, but early maturing species were still able to achieve GY up to 1.6 t ha⁻¹ if they had high TSW (≥ 164 g) and LNC above 40 mg g⁻¹. In addition, plant traits which are determinant for good performance in one property could be disadvantageous for another when they are combined with other traits. For example, long crop duration is in favor of GY and %Ndfa but could end up to 80% YR under weed infestation among species with large leaflets. It is therefore difficult to define one ideal combination of plant functional traits which would maximize all ecosystem properties. TSW participated in the prediction of most agro-ecosystem properties. However, we found that prediction of ecosystem properties did not rely on the central traits used for the quantification of each axis alone. Leaflet length was found more relevant than plant height to predict competitive ability against weeds and days to maturity a better predictor than SLA for prediction of pulse productivity as indicated by CART results for prediction of biomass and grain yield performances.

The first targeted ecosystem service was food production under dry conditions. The objective was not to differentiate pulse species by their drought resistance but rather by their ability to maintain high production rate under drought, which is a more relevant trait for food production. WUE is a good candidate for that purpose even though it is not the only driver of effective use of available water or drought resistance. Over the 28 species considered here, high WUE was observed mainly for species with high LNC. LNC at anthesis and grain yield under drought have been previously found to be strongly linked (Borrell and Hammer, 2000). Indeed, when nutrient uptake is limited by water availability (Chapin, 1993), remobilization of N from vegetative tissues becomes particularly important for grain growth (Ta and Weiland, 2010). Moreover, high LNC is usually associated to the “stay green” type. More especially in case of terminal drought, it has been shown that if LNC declines to a critical threshold, leaf senescence will set up (Borrell and Hammer, 2000; Borrell, Hammer and Van Oosterom, 2001). It is therefore not surprising that N leaf status is very closely related to the longevity of photosynthetic organs (Kikuzawa, 1991; Reich, Walters and Ellsworth, 1997). In case of terminal drought, high LNC maintains photosynthetic capacity for longer, sometimes leading to higher grain yield, and allows greater N remobilization. Furthermore, species with low LNC were more likely to have high WUE if associated with large leaf area. Although large leaf area may cost more water loss, it is also possible that it would result in more remaining photosynthesis area at the end of the drought stress period, thus allowing to achieve higher yields. WUE was poorly represented in the PCA. Because of this positioning, it could hardly be associated with other properties. Understanding its interaction with grain yield could have been particularly interesting for breeding purposes. Grain yield was not predicted with the same set of traits than WUE except for LNC (LNC is important for pod filling whatever water conditions are). Seed weight (TSW) was a good predictor of grain yield. Indeed, seed size is expected to be positively correlated with seedling biomass (Tamet

et al., 1996; Fayaud *et al.*, 2014), plant height and reproductive effort (Chapin, Autumn and Pugnaire, 2002). Seed size might also have been a result of agronomic selection such as erect habit, characteristic of most high yielding species and a trait highly related to domestication (Smartt, 1978a).

Cropping systems that incorporate grain legumes have been shown to strongly decrease N fertilizer rates (by 13–30% for wheat and 49–61% at the rotation level) through nitrogen fixation (Plaza-Bonilla *et al.*, 2017). However, legume species are not equivalent in their ability to supply exogenous nitrogen to the system. In our study, the percentage of nitrogen fixed varied substantially across species (from 23.75 ± 15.34 % to 93.33 ± 3.51 %). In addition to species inherent capacity to fix N, this variation could be attributed to nutritional factors, environmental conditions, rhizobia strains or host characteristics (Reichardt *et al.*, 1987; Nambiar, Rupela and Kumar Rao, 1988; Hardarson *et al.*, 1993). Since the data collected focused on plant traits, an incomplete picture of what drives nitrogen supply might have been obtained. CART regression showed that high nitrogen supply was mostly achieved by late maturing species. This result is in accordance with previous studies (Kumar Rao and Dart, 1987; Piha and Munns, 1987). Nitrogen fixation is more favorable for grain yield during the latter part of the growth cycle of a legume than it is during early growth (Piha and Munns, 1987; Vance, 2011). These factors account for the superior symbiotic performance of late maturing bean cultivars. In addition, any process that increases growth rate also increases tissue turn-over and loss of carbon, nutrients and water, alongside with decreasing allocation to storage (Grime *et al.*, 1997a) and, thus, possible allocation to nodules. Our results highlighted small leaflets as a secondary trait involved in high nitrogen supply. The presence of compound leaves is a widespread trait of legume species and leaves divided into small leaflets appears to be a frequent component of ecological strategies emphasizing a productive photosynthetic apparatus (Leavitt, Dobrenz and Stone, 1979; McKey, 1994a). In theory, nitrogen fixation may require/mobilize 10 to 20% of the total plant photosynthesis (Schubert, 1981; Vance, 2011). Thus, high photosynthetic productivity might allow a greater allocation of photosynthetic compounds to nodules. N₂ fixation has been shown to be closely synchronized with the rate of supply of translocate from the shoot to nodules (Tissue, Megonigal and Thomas, 1997; Herridge and Pate, 2008). Here we considered the proportion of nitrogen fixed by a plant (%Ndfa), independently of biomass production aspect, as an indicator of nitrogen supply. This trait is related to plant N fixation efficiency and its ability to grow in nitrogen-poor environments. However, biomass yield is hardly dissociable from nitrogen fixation as it is a process driven by N demand (Hartwig, 1998; Hartwig *et al.*, 2002). CART regression showed that high biomass species had large SLA. High SLA might allow (given favorable growth conditions) a shorter payback time on a gram of dry matter invested in a leaf (Poorter, 1994), therefore improving production rate.

The last targeted ecosystem service was competitiveness toward weeds, which is a service that is particularly relevant when considering legumes as cover crops (Kruidhof, Bastiaans and Kropff, 2008) or growing grain legumes without herbicide use. In addition to providing an indicator for weed

control, competitiveness towards weeds might also be a good indicator for the management of plant species interactions in intercropping (Fan *et al.*, 2006). Higher level of competition, however, might also induce competition for nutrient or water supply (Craine and Dybzinski, 2013). Our results show that species with a higher WUE were more insensitive to weed competition. It has also been shown in previous studies that root competition for soil resources and shoot competition for light are occurring simultaneously and are interrelated in pea-barley intercropping (Corre-Hellou, Fustec and Crozat, 2006). Yet, in our study, %YR was mostly found correlated with LAI indicating that light competition might be more important. Many ecological studies have pointed out that competition is mostly encountered in highly productive environments. Indeed, when soil resources are more abundant, light might become the limiting resource (Gaudet and Keddy, 1988). LAI is higher for large seeds with hypogeal germination. As for biomass, grain yield and nitrogen fixation, LAI can be related to seedling vigor. In addition, our results show that yield was less likely to be impacted by weeds for early maturing species with small leaves; which are also species with a high nitrogen fixation ability. Indeed, in our study, small leaflets allowed early maturing species to achieve almost similar %Ndfa level than late maturing species. This suggests that the use of alternative nitrogen source is also an important component of pulses competitive ability. Some studies highlighted that nitrogen fixation increased in case of interaction with non-fixing species (Nesheim and Boller, 1991; Fan *et al.*, 2006) suggesting competition for nitrogen might disfavor pulse species which do not have the capacity to improve their fixation rate. In addition, small leaves are less expensive to produce (Poorter, 1994) and might be more efficient for light interception as it reduces the risk of auto-shading (Smartt, 1978a). Beside leaflet size, early growth provides an important advantage to the crop for light acquisition as well as for nitrogen accumulation through a faster and deeper root growth (Bedoussac and Justes, 2010b).

IV. Limits

The approach followed in this study has some limitations, which could potentially limit the extent of our conclusions. Due to the relatively low and uneven number of values reported across traits and species, we had to consider aggregated values for each species and each trait. After comparing different summary statistics, mean was found to be the best aggregating function (compared to median, maximum, and minimum). Standard deviation was judged non reliable to estimate the intraspecific variation, due to the relatively low and uneven number of values for evaluating intra-specific variation. However, for each documented species, trait and agro-ecosystem properties have been measured on various genotypes or cultivars, which probably accounts for within species variability. In addition, genera boundary are still discussed in grain legumes. In this study, we relied on current taxonomy to delimitate species, which may have misled us into aggregating together trait values that actually belong to distinct species. This inaccuracy in identifying species may have contributed to some extent to blur limits between identified plant strategies. Data availability also led

to some degree of incomplete database where some traits or species were more documented than others. We tried to overcome this weakness by imputing data through probabilistic principal component analysis, although we acknowledge that this estimation might have induced bias for the agro-ecosystem property's prediction. Although non-optimal data availability and accuracy may explain partly the large number of trait combinations predicting each agro-ecosystem property, our analysis was solid enough to identify ubiquitous contributing traits and plant strategies for each targeted ecosystem service.

E. Conclusion

Defining crop ideotypes through trait profiles for ecosystem service provision is tempting. However, our study suggests that the numerous correlations between traits and between agro-ecosystem properties themselves, and more importantly the inconsistency of trait combinations that best predict the different properties, may hinder it. Our analytical framework suggests that variation in agronomic performance and related ecosystem services, at an interspecific level among pulses, follow ecological strategies theories. However, further work will be required to explicitly take into account the effects of environmental changes as well as the role of intraspecific variation in such a diverse group. Although agro-ecosystem properties were predicted by multiple alternative combinations of traits it was possible to identify leaflet length, days to maturity, seed weight, and LNC as ubiquitous plant traits that discriminate ecosystem services provisioned by pulses. This discrimination was more or less consistent with adaptive strategies schemes since advantageousness of a trait was dependent on the ecosystem service under consideration. The achievement of a desired set of ecosystem services may require combining antonymic or negatively correlated properties which renders the identification of suitable traits pattern more complex, as more combinations become susceptible to be suitable.

F. Supplementary materials

Supplementary table S 1 : 43 species documented in this study

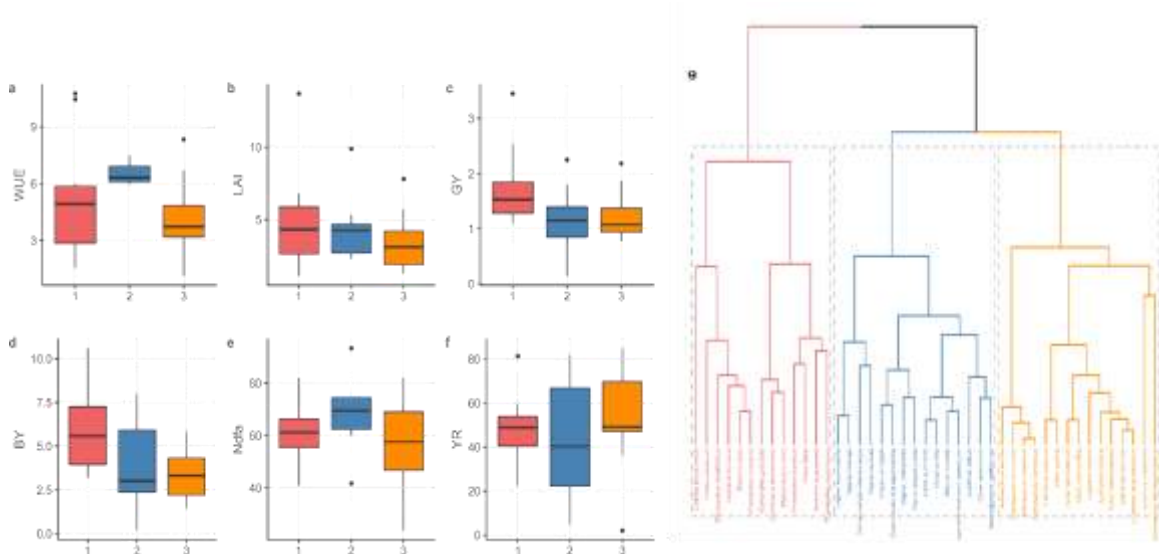
Species	abbreviation	Species	abbreviation
Arachis hypogaea	Ahy	Mucuna pruriens	Mpr
Astragalus annularis	Aan	Phaseolus acutifolius	Pac
Astragalus boeticus	Abo	Phaseolus coccineus	Pco
Astragalus edulis	Aed	Phaseolus lunatus	Plu
Astragalus hamosus	Aha	Phaseolus vulgaris	Pvu
Cajanus cajan	Cca	Pisum humile	Phu
Canavalia ensiformis	Cen	Pisum sativum	Psa
Canavalia gladiata	Cgl	Psophocarpus tetragonolobus	Pte
Cicer arietinum	Car	Sphenostylis stenocarpa	Sst
Cicer reticulatum	Cre	Trigonella foenum-graecum	Tfo
Cyamopsis tetragonoloba	Cte	Vicia ervilia	Ver
Glycine max	Gma	Vicia faba	Vfa
Lablab purpureus	Lpu	Vicia monantha	Vmo
Lathyrus cicera	Lci	Vicia narbonensis	Vna
Lathyrus ochrus	Loc	Vicia sativa	Vsa
Lathyrus sativus	Lsa	Vigna aconitifolia	Vac
Lens culinaris	Lcu	Vigna angularis	Van
Lupinus albus	Lal	Vigna mungo	Vmu
Lupinus angustifolius	Lan	Vigna radiata	Vra
Lupinus luteus	Llu	Vigna umbelatta	Vum
Lupinus mutabilis	Lmu	Vigna unguiculata	Vun
Macrotyloma uniflorum	Mun		

Supplementary table S 2 : Pearson correlation between 6 agro-ecosystem properties. See Table 2 for abbreviations.

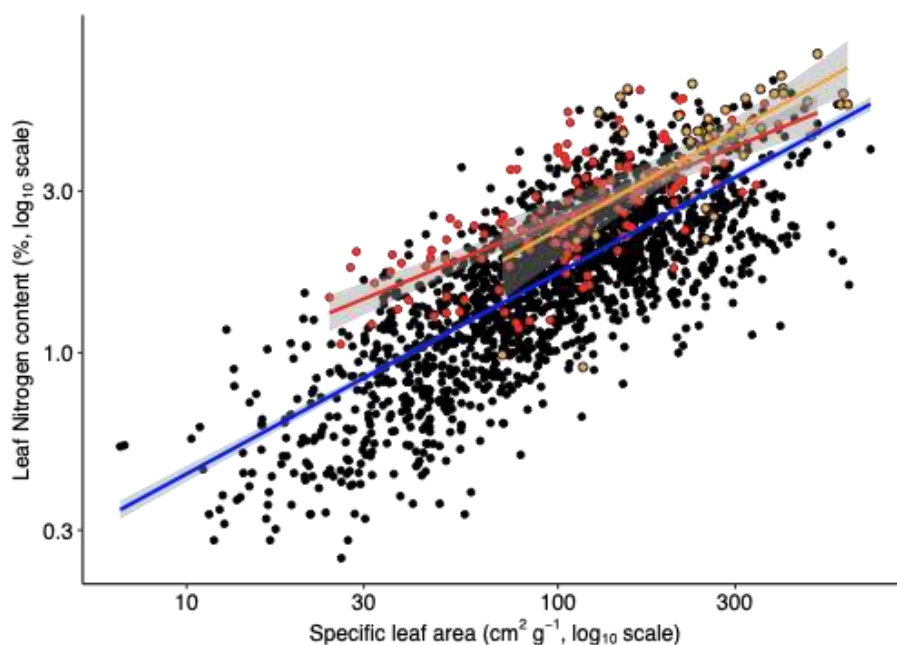
	Grain yield	Biomass	LAI	Ndfa	Yield reduction
Grain yield					
Biomass	0.69***				
LAI	0.02	0.35			
Ndfa	0.13	0.22	0.33		
Yield reduction	0.27	0.09	-0.15	0.04	
WUE	0.07	0.27	0.35	0.09	-0.07

Supplementary table S 3 : Pearson's correlation coefficients between 15 functional traits. See Table 1 for abbreviations

	DM	DF	Seed_size	lflt_nb	lflt_lth	lflt_wth	PH	TSW	LNC	Seed_oil	Seed_prot	LA	SLA	S/R
DM														
DF	0.7***													
Seed_size	0.16	-0.11												
lflt_nb	0.25	0.23	-0.34											
lflt_lth	-0.15	-0.25	0.5***	-0.81***										
lflt_wth	-0.23	-0.34*	0.43***	-0.73***	0.9***									
PH	0.25	0.11	0.46**	-0.45**	0.6***	0.55***								
TSW	0.14	-0.12	0.87***	-0.35	0.51***	0.46**	0.47**							
LNC	-0.12	-0.04	0.1	-0.18	0.09	-0.01	0.22	0.17						
Seed_oil	0.13	-0.16	0.37*	0.09	0.06	0.06	0.03	0.34*	-0.12					
Seed_prot	0.26	0.13	-0.18	0.26	-0.24	-0.32*	-0.15	-0.19	0.21	0.45**				
LA	-0.1	-0.13	0.42**	-0.56***	0.61***	0.55**	0.44**	0.46**	0.26	0.43**	0.04			
SLA	-0.22	-0.2	0.36*	-0.22	0.24	0.13	-0.05	0.29	0.24	-0.12	-0.12	0.05		
S/R	0.03	0.08	0.09	-0.33*	0.5***	0.52**	0.29	0	-0.4**	-0.09	-0.36*	0.1	-0.15	
HI	0.03	0	0.18	0.26	-0.31*	-0.25	-0.26	0.14	-0.09	0.14	0	-0.17	0.25	-0.24



Supplementary figure S1 : Cluster's values over each property considered: water use efficiency $\text{kg ha}^{-1} \text{mm}^{-1}$ (a), leaf area index (b), grain yield t ha^{-1} (c), biomass yield t ha^{-1} (d), nitrogen derived from atmosphere % (e), yield reduction due to weeds % (f) and hierarchical clustering of the 43 pulses species based on the five most important traits for properties predictions (SLA, LNC, TSW, DM, leaflet length) (g)



Supplementary figure S2 : Relationship between specific leaf area and leaf nitrogen content across wild species (Wright *et al.* 2004; black dots), nitrogen fixing species (Wright *et al.* 2004; red dots) and nitrogen-fixing pulses species (this study; orange dots). Lines are linear regression lines for each dataset, respectively.

Contributions

JG gathered the bibliographic material, performed the statistical analysis and led the writing, DV and HM had the idea of the study, directed the analysis and contributed to writing, MEG and JW contributed to writing the manuscript

Acknowledgements

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G. References

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**CHAPTER 3. *FUNCTIONAL DIVERSITY OF PULSES'*
SPECIES IN RELATION WITH AGRO-ECOSYSTEM
*PROPERTIES***

Functional diversity of pulses' species in relation with agro-ecosystem properties

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ABSTRACT

Crop diversity management in agriculture is a fundamental principle of agroecology and a powerful way to promote resilient and sustainable production systems. Pulses are especially relevant for diversification issues. Yet, the specific diversity of legumes is poorly represented in most cropping systems. We used the trait-based approach to quantify the functional diversity of 30 crop pulses varieties, belonging to ten species, grown under common field conditions. Our aim was to test relationships between traits, yield, and supporting agro-ecosystem properties. Our experimental results highlighted trade-offs between agro-ecosystem properties supported by different legume species. Also, results demonstrated the relevance of leaf nitrogen content, leaf area ratio, and reproductive phenology to predict most of the trade-offs observed between agro-ecosystem properties. A comparison of these experimental data collected within a uniform environment with a previous analysis based on literature data collected in diverse agronomic situations suggested that some traits are more plastic than others and therefore contribute differently to frame legumes diversity depending on the conditions of observation. Present results suggested that the implementation of such trait-based approach would rapidly benefit the selection of species/varieties for specific targeted agro-ecosystem services provisioning under specific (environmental or management) conditions.

A. Introduction

Crop diversity management in agriculture is a fundamental principle of agroecology and a powerful way to promote resilient and sustainable production systems (Gaba et al., 2015). Agro-ecosystem management provides multiple alternatives to monoculture, such as intercropping or rotation. Pulses are especially relevant for those diversified cropping systems. Their well-known ability to fix atmospheric nitrogen reduces fossil energy consumption and makes them particularly suitable for low-input systems. They are also particularly important in human nutrition as a source of proteins to complement cereal-based diets (Singh et al., 2003; Tharanathan and Mahadevamma, 2003). Yet, the specific diversity of legumes is poorly represented in most cropping systems. While more than 80 pulses species are known to contribute to human diet, the FAO database includes only 11 of them (Tiwari et al., 2011). This lack of attention creates a risk of continued erosion of knowledge and genetic resources that could further hamper the integration of these crops into more diversified cropping systems (Dansi et al., 2012).

To increase diversity within cropping systems, choosing appropriate species is an important prerequisite (Brooker et al., 2015). To address this concern, the trait-based approach is well suited for designing diversified cropping system capable of providing specific sets of services. In ecology the approach has been increasingly employed to link biodiversity to ecosystem functioning and services (Lavorel and Garnier, 2002; Violle et al., 2007; Garnier and Navas, 2012); a methodology that could be applied to cropping systems (Litrice and Violle, 2015; Martin and Isaac, 2015; Wood et al., 2015; Damour et al., 2018). This approach could help to identify possible strategies by which multiple agro-ecosystem properties can be jointly determined for a better ecosystem services provision, which is a crucial aspect of agro-ecosystem management. However, trait-based approach requires extensive data collection and gathering to develop trait databases for crop species or varieties and document relationships between traits and services (Barot et al., 2017). As a neglected group of species, pulses are particularly difficult to document. As a consequence, data availability is often a limiting factor for implementing a trait-based approach (Barot et al., 2017), especially on pulses.

In a previous study, Guiguitant et al. (2020) elaborated a database suitable for functional trait approach on pulses. The database gathered disparate data from multiple published and/or public sources and contained data on plant functional traits for 43 pulse species, and agro-ecosystem properties recorded at canopy level for a subset of these species. The analysis the functional trait space highlighted three ecological strategies among cultivated pulses, that were similar to those already described in spontaneous species in the Leaf-Seed-Height (LSH) scheme (Westoby, 1998). However, reduced data on agroecological properties and data disparity did not allow to identify clear link between traits and agro-ecosystem properties. In an applied perspective of supporting the integration of pulses in cropping systems, a consolidated analysis of trait-properties relationships is

still needed to identify which traits would make legume species more productive, competitive or adapted to low nitrogen or water conditions and favor production services. Trade-offs between plant traits and how they determine the different strategies also require more attention.

In the current study, a complementary dataset was collected from an experimental set up, in which 30 pulse varieties, belonging to ten species, were monitored in similar environmental conditions. In accordance with the previous study by Guiguitant et al. (2020), agro-ecosystem properties are defined in this study as the result of processes measured or calculated at the canopy level (Garnier et al., 2007), such as biomass or leaf area index. Conversely, functional traits (Violle et al., 2007) designate characteristics of an individual plant or plant organ, which can be morphological, physiological or phenological and that are supposed to have direct or indirect effects on agro-ecosystem properties. The aim of this study was to use a trait-based description of the functional diversity of pulses species to explicit relationships between traits, yield and supporting agro-ecosystem properties. This study also aimed at assessing the effect of trait measurement conditions on trait values and whether it could distort the relations between traits and agro-ecosystem properties. To do so, analyses were compared when using experimental data collected within a uniform environment and literature data collected in diverse agronomic situations.

B. Material and methods

I. Plant material and growing conditions

A field experiment was conducted at UCOSSEM's experimental farm in Lectoure (Southern France) on a deep clay-limestone soil. Three varieties of ten pulses species (Table 3) were planted in two sequential batches/sub-experiments on contiguous fields, according to their temperature requirements: Cool season legumes were sown after the last negative temperatures, on 14 March 2018, and warm season legumes were sown on 7 June 2018, when daily temperature was over 15°C. Each batch/sub-experiment was laid out in split plot design with four replicates, varieties being subplots and species the main plot. In the case of *Vigna acognitifolia*, *Vigna mungo*, and *Vigna unguiculata*, a permutation between varieties of the three species was possibly done in the experimental layout as provided varieties were tagged as "types" for each species. For both sub-experiments, preceding crop was sunflower, and fields were fertilized with 0/22/8 NPK (350 kg ha⁻¹) fertilizer before sowing. Each species was manually inoculated before sowing with an adequate strain of rhizobia (provider: UMR LSTM, Montpellier, France). Sowing density was chosen according to agronomical recommendations for each species and ranged from 10 to 150 plant m⁻². Manual weeding and drip irrigation were performed on a regular basis from sowing. Soil moisture was recorded weekly in each block and for each species at three depths (0-20, 20-40 and 40-60 cm) using TDR probes installed vertically at sowing (mini TRASETM with buriable

probes, Soilmoisture Equipment Corp., CA) and confirmed that the plants did not experiment any water deficit in none of the two sub-experiments.

Table 3 : List of the ten species included in the experiment with a description of the three varieties associate to each species the abbreviation used in the study, sources of the seeds and the β value used for %Ndfa computation.

Species	varieties	Abb.	Source	β (‰)
<i>Vigna mungo</i>	ISRA 58-77 (V1), ISRA 66-41 (V2), ISRA 67-30 (V3)	Vmu	ISRA, Sénégal	-1,75
<i>Vigna unguiculata</i>	YACINE (V1), SAM (V2), IT98K-1092-1-1 (V3)	Vun	ISRA, Sénégal	-1,61
<i>Vigna aconitifolia</i>	SH_VM (V1), SH_VA (V2), Tvu 45 35 (V2)	Vac	ISRA, Sénégal	-0,91
<i>Lotus tetragonolobus</i>	Landrace	Lte	Palmbeach medicinal herb, rarexoticseed, hobbyseed	-0,12
<i>Phaseolus vulgaris</i>	Red kidney (V1), zorro (V2), michelet (V3)	Pvu	Epi de Gascogne, France	-2,16
<i>Cicer arietinum</i>	Elmo (V1), billy bean (V2), orion (V3)	Car	Epi de Gascogne, France ; USDA Pullman, WS	-1,75
<i>Lens culinaris</i>	Belezana (V1), Richela (V2), Anicia (V3)	Lcu	Epi de Gascogne, USDA Pullman, WS	-0,56
<i>Trigonella foenum-graecum</i>	FENUSOL, landrace	Tfo	Epi de Gascogne, France ; ICARDA, Morroco	-0,42
<i>Lathyrus sativus</i>	Wassie (V1), Almenaza (V2), Chicharo (V3)	Lsa	Epi de Gascogne, France	-0,38
<i>Vicia faba</i>	Aguadulce (V1), alfia (V2), lobaba (V3)	Vfa	ICARDA, Morroco	-0,5

II. Measurements of individual plant traits

Occurrence of phenological stages (date of 50% flowering, date of physiological maturity), were recorded in calendar days, then converted into thermal units using a base temperature of 0°C for *C. arietinum* and *V. faba*, 2°C for *L. culinaris*, 3.5°C for *L. sativus*, 4.1°C for *T. foenum-graecum* (Covell et al., 1986; Lamichhane et al., 2020), 4.7°C for *L. tetragonolobus* (Moot et al., 2000), 6°C for *P. vulgaris* (López et al., 2003) and 8.5°C for *Vigna sp.* (Craufurd et al., 1996). This allowed to compute degree days from sowing to flowering (FLO) and degree days from sowing to maturity (MAT).

Biomass was harvested at flowering stage and final harvest at seed set. At each sampling date, plants were collected on 0.5 m² quadrats, in the central rows to avoid border effect. Plant number was recorded, then leaves, stems, flowers and pods were detached, then dried separately at 60°C until weight stability and weighed. Leaf and stem dry mass ratio over total aboveground plant biomass (LMR and SMR, respectively) were calculated at flowering, and harvest index (HI) was

calculated at maturity. Leaf area ratio (LAR; total plant leaf area over total aboveground biomass), total leaf area (LA, $\text{mm}^2 \text{ plant}^{-1}$), specific leaf area (SLA, $\text{cm}^2 \text{ g}^{-1}$), and leaflet length (LL, mm) as well as leaflet width (LW, mm) were measured at flowering, whereas thousand seed weight (TSW, g) was determined at harvest.

Total nitrogen content was determined (Vario PyroCube auto-analyzer and Precision mass spectrometer) on leaves, stems, and grain samples collected at maturity and at flowering. Total plant nitrogen content was computed both at flowering and maturity. Leaf nitrogen content (LNC, mg g^{-1}), and seed nitrogen content (SNC, mg g^{-1}) were determined at flowering and at maturity, respectively.

Phyllochron was calculated from observations of three tagged plants on each plot for which emerged leaves were counted weekly (cool season legumes) or twice a week (warm season legumes).

The rate of soil coverage was estimated by measuring soil cover weekly, using nadiral photos taken at the same location within each plot, with a large angle camera at chest height, then analyzed with ImageJ software (Schneider et al., 2012). Soil cover was modeled as a logistic function of thermal time. The maximal rate of soil coverage was defined as the slope at the inflexion point of the logistic curve.

III. Measurement of agro-ecosystem properties

Agro-ecosystem properties were measured on the same experimental plots (Table 4). Grain yield (GY, t ha^{-1}), biomass yield (BY, t ha^{-1}) and biomass measured at flowering (t ha^{-1}) were determined from plant sampling on 0.5 m^2 quadrats. Water use efficiency (WUE) was computed as grain mass produced per mm of water input (rainfall and irrigation). Maximum soil cover (%), leaf area index (LAI) and the duration (in thermal units) of the phase when soil coverage is above 80% of the maximum (soil cover duration) were recorded. Finally, the capacity to fix nitrogen from the atmosphere was estimated through the percent of nitrogen derived from biological fixation at flowering and at maturity, measured with the natural isotopic dilution method, using barley and maize grown in the experiment outer borders as control plants for cool season and warm season legumes, respectively. From the ^{15}N abundance ($d^{15}\text{N}$) of the samples the proportion of plant N derived from atmosphere (%Ndfa) was calculated from the equation 4 (Shearer and Kohl, 1986):

$$\%Ndfa = 100 \frac{\delta^{15}N_{ref} - \delta^{15}N_{legume}}{\delta^{15}N_{ref} - \beta} \quad (\text{Equation 4})$$

Where β is the ^{15}N natural abundance of the N derived from BNF in the aerial tissue of the legume, $\delta^{15}N_{ref}$ is the ^{15}N abundance in the control plant (maize or barley) tissues, and $\delta^{15}N_{legume}$ is the ^{15}N abundance in legume tissues. The β values used are detailed in Table 3 (Unkovich et al., 2008)

Table 4 : List of agro-ecosystem properties and traits measured in the experiment with their respective abbreviation and unit.

Variable	Abbreviation	unit
<i>Agro-ecosystem property</i>		
Grain yield	GY	t ha ⁻¹
Biomass yield	BY	t ha ⁻¹
Leaf area index	LAI	
% Nitrogen derived from atmosphere at maturity	Ndfa_MAT	%
% Nitrogen derived from atmosphere at flowering	Ndfa_FLO	%
Water use efficiency	WUE	kg ha ⁻¹ mm ⁻¹
Biomass at flowering	B_FLO	t ha ⁻¹
Maximal soil cover	maximum soil cover	%
Soil cover duration	Soil cover duration	Thermal unit
<i>Traits</i>		
Leaf area	LA	mm ²
Specific leaf area	SLA	cm ² .g ⁻¹
Leaflet length	LL	mm
Leaflet width	LW	mm
Leaf nitrogen content	LNC	mg.g ⁻¹
plant N content at flowering	Nplant_FLO	g.plant ⁻¹
plant N content at maturity	Nplant_MAT	g.plant ⁻¹
Phyllochron	Phyllochron	Thermal unit
Soil cover rate	Cover rate	
Degree days to maturity	MAT	Thermal unit
Degree days to flowering	FLO	Thermal unit
Thousand seed weighed	TSW	g
Leaf area ratio	LAR	-
Leaf mass ratio	LMR	-
Stem mass ratio	SMR	-
Harvest index	HI	-
Seed nitrogen content	SNC	mg.g ⁻¹

IV. Data analysis

Data were first analyzed through the prism of the framework presented in Guiguitant et al. (2020) in order to compare the variability observed with the global functional diversity of pulses species. Data of the present field measurements were projected as supplementary individuals in a PPCA computed with nine common functional traits (DM, DF, HI, LL, LW, LA, SLA, LNC, and TSW) values taken from Guiguitant et al. (2020) database. We used Euclidian distance to quantify the distance between the scores of barycenter of each species tested in the field and previously published data. The consistency of species ranking in trait values across literature and experimental data was tested with Spearman's rank correlation analysis.

Second, a principal component analysis (PCA) was performed on experimental data including all traits measured. Agro-ecosystem properties were added as supplementary variables, in order to investigate how they fit into the phenotypic space defined by the traits. The appropriate number of PCs was determined with Kaiser criterion. A between and within-class correspondence analysis was carried out to evaluate the percentage of variance captured by the intra and inter-specific diversity.

Finally, trade-offs among agro-ecosystem properties were analyzed using a hierarchical clustering analysis (HCA). A classification and regression tree (CART) analysis was done on raw data ($n = 120$) to identify how functional traits could allow to predict the clusters of varieties obtained from the HCA. Regression trees are prediction models obtained through machine-learning algorithms that recursively partition the data space in order to fit the simplest prediction model within each partition. The resulting partitioning can be represented graphically as a decision tree. The model was first trained through a bagging procedure which consists in generating training subsets of individuals using bootstrap sampling to generate independent tree models. Each tree was tested with the individuals discarded from tree construction and the tree that minimize the "out-of-bag" error was chosen. The random forest aggregation method, which consists in adding a random component to the choice of variables in the model was also used. This method does not allow to generate a visual decision tree but it provides indices proportional to the importance of each variable in the aggregated model and thus its participation to the discrimination between groups of individuals (here, varieties). Mean Decrease in Gini impurity (MDG) is a forest-wide weighted average of the decrease in the Gini impurity metric between the parent and daughter nodes that a variable is splitting. It can be defined as the total decrease in node impurity (weighted by the proportion of samples reaching a given node) averaged across all of the trees that make up the forest. The most important variables to the model will have the largest MDG values, conversely, the least important variable will have the smallest MDG values.

All statistical analyses were performed in the computing environment R 3.5.2 (R Core Team, 2018) using *pcaMethods* package (Stacklies et al., 2007), *rpart* package (Therneau et al., 2015), *ade4* package (Dray and Dufour, 2007) and *randomForest* package (Breiman, 2001).

C. Results

I. Consistency of functional trait space

A first PPCA was performed on the 43-species-database from Guiguitant et al. (2020) using only traits that were also measured in the present field experiment. The first three PC axes of this PPCA explained 70% of total variance observed in the literature (Figure 10). PC1 (34%) was associated with morphological traits, especially leaf traits (LL, LW, LA and to a lesser extent TSW). PC2 (24%) was positively associated late flowering (DF) and maturity (DM) and to a lesser extent HI. PC3 (12.4%) was strongly associated to SLA and LNC. Experimental data variability on PC axes was in the same range as the literature variability. The variances of individuals score from the present experiment were similar to the variances of the scores of active individuals from the literature on the first two PCs. Experimental variance was close to 100% and 60% of literature variance on PC1 and PC2, respectively. On the contrary, the variance of supplementary individuals on the third axis (driven by SLA and LNC) was almost three times higher than the variance of active data from the literature. This could be attributed to LNC which range was larger in the experimental sample (10 to 60 mg g⁻¹ against 19 to 60 mg g⁻¹; Supplementary figure S3) while SLA range was similar between the two data set. For all other traits, field measurements range was always narrower than the one observed in Guiguitant et al. (2020), for a larger set of species.

Another outcome of this comparison was that the barycenter of each species tested in the present experiment was very close to its equivalent from the literature database. Distance between the two corresponding barycenters was especially short in the first plane of the PPCA (Figure 10). Species ranking in literature versus present data remained conserved on PC1 ($\rho = 0.85$, $p = 0.003$) and to a lesser extent on PC2 ($\rho = 0.53$, $p = 0.10$). Species rankings were rather inconsistent on PC3 ($\rho = 0.24$, $p = 0.12$). Cool season species had barycenters closely positioned along the three dimensions of the PPCA (Euclidian distance lower than 2; Table 5). Among them, *C. arietinum* and *T. foenum-graecum* had the highest Euclidian distance mostly because of the divergence with literature on the third axis, while *V. faba* diverged mostly on the second axis. For warm season species, distances between barycenters from the literature and from the present experiment were larger, on PC3 but also on PC2 for *Vigna* sp.

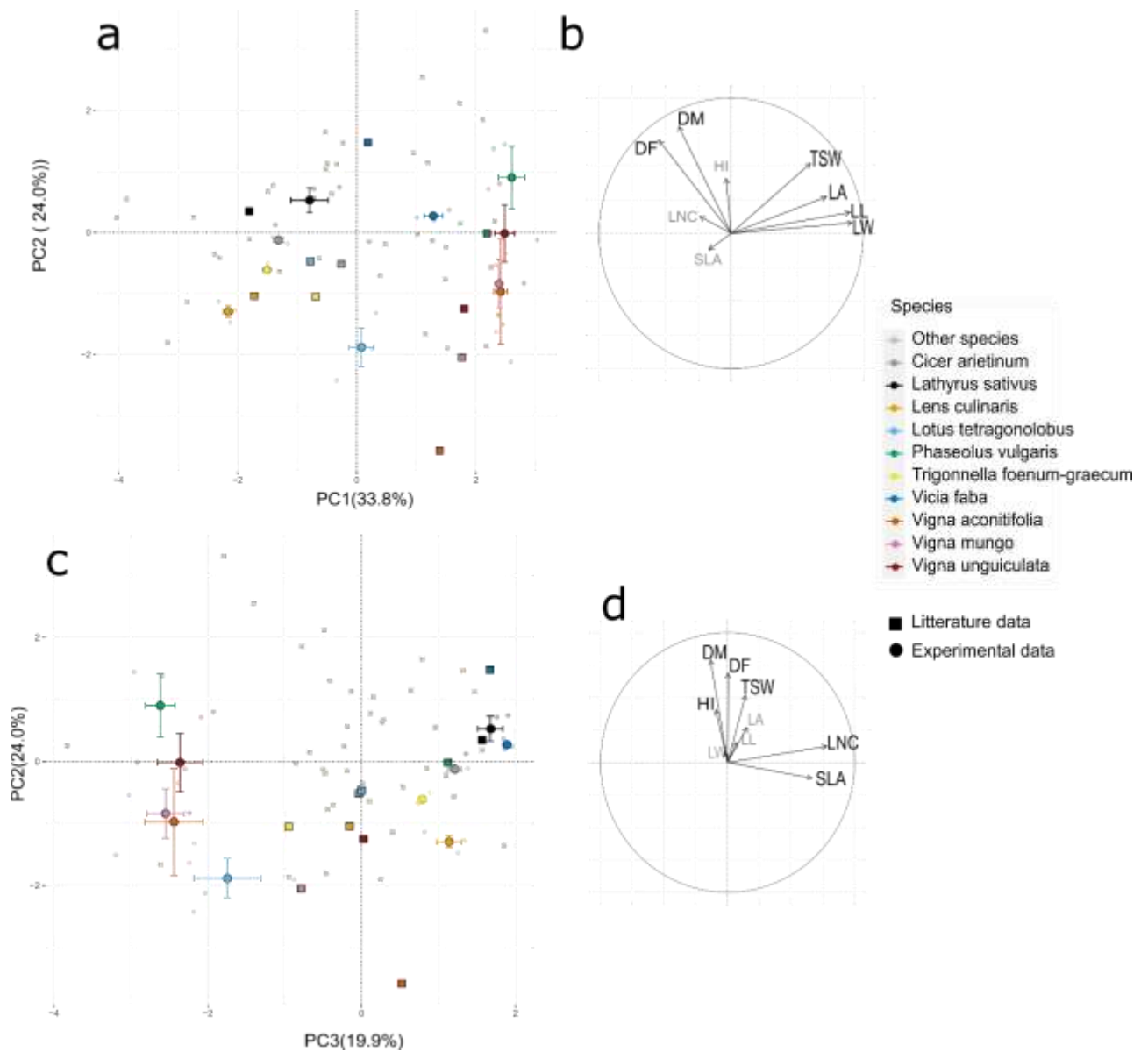


Figure 10 : Principal component analysis (PCA) performed on 9 functional traits collected 43 pulses species. (a, c) Individual species projection on the first three axes of the PCA, light grey points represent the 43 species issued from literature (Guiguitant et al., 2020) and colored points are the data issued from the current experimental study projected as supplementary individuals. Transparent points are the data of the four plots for the three varieties of the ten species, the opaque points represent the barycenter of each species and the horizontal and vertical bar the standard deviation along axes. Visualization of the variables and correlation circles on (b) PC1-PC2 and (d) PC2-PC3 planes. See Table 4 for abbreviations of traits.

Table 5 : Euclidian distance between literature and current experimental study coordinates on the firsts three PCA axes of the barycenter of each species

	PC1	PC2	PC3	PC123
Cicer arietinum	1.1	0.39	1.25	1.68
Lathyrus sativus	1.0	0.18	0.11	1.03
Lens culinaris	0.4	0.25	1.29	1.39
Lotus tetragonolobus	0.9	1.41	1.74	2.40
Phaseolus vulgaris	0.4	0.92	3.74	3.87
Trigonella foenum-graecum	0.8	0.44	1.73	1.97
Vicia faba	1.1	1.20	0.22	1.64
Vigna aconitifolia	1.0	2.61	2.96	4.08
Vigna mungo	0.6	1.21	1.76	2.23
Vigna unguiculata	0.7	1.23	2.39	2.77

II. Characterization of functional trait diversity

The first four axes of the PCA performed on 30 individuals and 16 traits from the present experiment (Table 4) captured 80% of the total variance (Figure 11). Interspecies variance explained 81% of the total variance while intra-species explained 19%.

Leaf attributes were strongly associated with PC1 (40%). PC1 opposed LA, LL and plant N content at flowering to SLA, LNC, SNC and LAR. This axis strongly opposed cool season species which are characterized by small leaflets and small LA with high leaf and seed nitrogen content and a large LAR, to warm season species characterized by large leaflet, high LA and high plant nitrogen content. PC2 (20%) opposed LMR to SMR and to a lesser extend cover rate. The cover rate was significantly negatively correlated to MAT ($r = -0.72$, $p < 0.001$). SMR was also significantly negatively correlated to the phyllochron ($r = -0.60$, $p < 0.001$). *L. tetragonolobus* and *P. vulgaris* were apart from all other species on PC2, and both expressed a high LMR at flowering. PC3 (11%) and PC4 (9%) were associated to FLO and TSW, respectively. PC3 opposed *V. faba* to *L. culinaris* and *L. tetragonolobus*, whereas PC4 opposed *C. arietinum* to *P. vulgaris*.

All agro-ecosystem properties, except LAI, biomass at flowering and Ndfa at maturity were not well represented in the factor planes formed by the fourth PCs constructed with traits (Figure 11). LAI and biomass at flowering were relatively well represented in the first plane and were significantly positively correlated to LL ($r = 0.46$, $p < 0.001$; $r = 0.63$, $p = 0.01$, respectively) and LA ($r = 0.67$, $p < 0.001$; $r = 0.83$, $p < 0.001$, respectively) as well as cover rate ($r = 0.59$, $p < 0.001$; $r = 0.60$, $p < 0.001$, respectively). Although Ndfa at flowering was poorly represented on the first PC, this property was significantly positively correlated to plant N content at flowering ($r = 0.41$, $p = 0.02$). Ndfa at maturity was not correlated to Ndfa at flowering ($r = 0.04$, $p = 0.83$) but was significantly positively

correlated to LNC ($r = 0.49$, $p = 0.005$) and SLA ($r = 0.57$, $p = 0.001$) as well as with SMR ($r = 0.43$, $p = 0.02$). The soil cover duration was opposed to LAI and biomass at flowering and significantly correlated with DDF ($r = 0.47$, $p = 0.009$) and to a lesser extent MAT ($r = 0.33$, $p = 0.08$). Maximum soil cover was not correlated to soil cover duration ($r = -0.03$, $p = 0.86$) and was slightly associated with SMR ($r = 0.35$, $p = 0.05$). Finally, GY, BY and WUE were associated to PC4 alongside TSW and BY was opposed GY and WUE on PC3 which was associated to FLO.

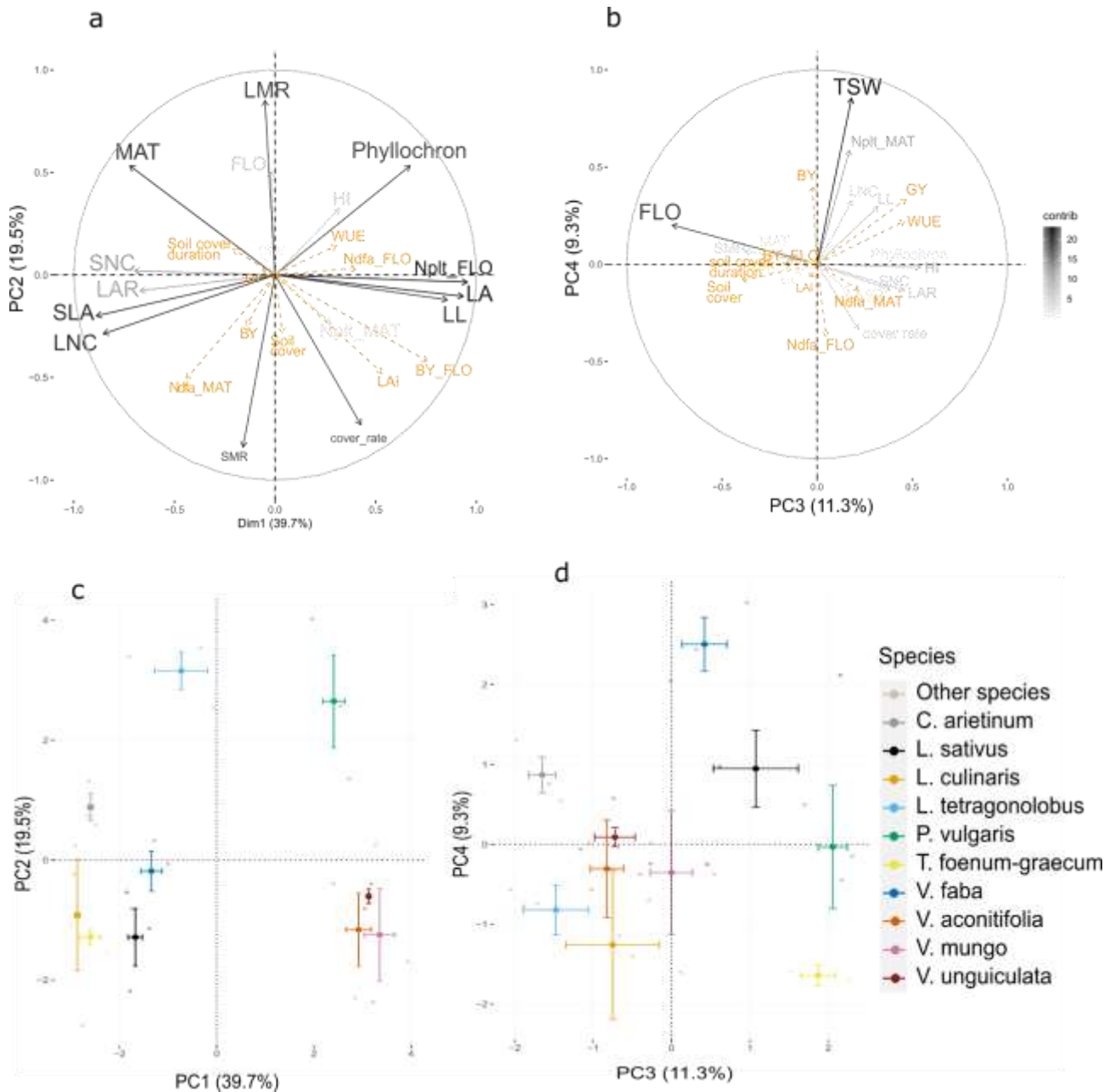


Figure 11: Principal component analysis (PCA) performed on functional traits measured on three varieties of ten species. Visualization of the variables and correlation circles on (a) PC1-PC2 and (b) PC2-PC3 planes. Gray scale represents the contribution of each variable to axes construction. Orange variable are agroecosystem properties, used as supplementary variables. See Table 2 for abbreviations of traits. Soil cover stands for Maximal soil cover

III. Trade-offs between agro-ecosystem properties

The classification analysis revealed seven clusters of species and varieties based on their agro-ecosystem properties (Figure 12). All varieties of a species generally fell into the same cluster. *V. acnitifolia* varieties were all in cluster 7 together with one variety of *V. unguiculata*. This cluster is apart from a group of close clusters composed essentially by cool season species; *L. sativus* varieties were grouped with *L. tetragonolobus* (cluster 2), *L. culinaris* was alone in cluster 3, cluster 5 was dominantly occupied by *T. foenum-graecum* and cluster 6 grouped both *V. faba* and *V. mungo*. These groups were distant from cluster 1 essentially represented by *C. arietinum* and cluster 4 that contained varieties of *P. vulgaris*, and of *V. unguiculata*.

Each cluster was characterized by a combination of values of agro-ecosystem properties. Cluster 1 grouped species with high production (GY and BY) as well as a high maximum soil cover and soil cover duration, but a low Ndfa and a low biomass and LAI at flowering. Cluster 2 was characterized by a low biomass at flowering as well as a low LAI at flowering and maximum soil cover but a high Ndfa at flowering and a long soil cover duration. Cluster 3 expressed the lowest yield as well as the lowest WUE but high Ndfa at maturity, a high LAI at flowering, and a long cover duration. Cluster 4 expressed the highest WUE concomitantly with a high GY and a low Ndfa at both stages. Varieties of cluster 5 were productive and expressed high Ndfa both at flowering and maturity. Yet, they showed a low maximum soil cover and soil cover duration. Cluster 6 was characterized by a low BY and GY, low Ndfa both at flowering and maturity, and poor soil covering capacity, yet LAI and biomass at flowering were high. Finally, cluster 7 was composed of varieties producing the highest biomass at flowering, along with the highest maximum soil cover, LAI and Ndfa at flowering.

IV. Relevant traits for prediction of agro-ecosystem properties and structuration of functional diversity

CART analysis showed very diverse trait combinations to predict the cluster affiliation. LNC was identified as the most important to discriminate the clusters trait as it had the highest mean decrease in Gini impurity (MDG) value, which means that it allowed to gain purity in the process of classification for most of the nodes in the CART it was associated to (Figure 13). LNC was assigned to the first division node and allowed to sort cluster 1 varieties apart from the remaining ones. Cluster 1 varieties have a LNC between 2.7 and 3 mg N g⁻¹. Varieties of cluster 4 were separated. Half of them fell on the same side of the tree as cluster 1 but exhibited lower LNC value (below 2.7 mg N g⁻¹) while the other half had an average LNC above 3 mg N g⁻¹. Varieties in this second cluster 4 half also had a LMR above 43% concomitantly with low plant N content at flowering and at maturity and a longer cycle. LMR, DDR and plant N content at maturity had strong MDG unlike the plant N content at flowering. Varieties with a LMR under 0.43 and having high MAT, were assigned either to cluster 3 (characterized by a high plant N content but only at flowering), or cluster 2 or 5 (both

characterized by high plant N content at flowering and maturity). Varieties in cluster 2 flowered later than those in cluster 5. Short cycle varieties were dominantly associated to cluster 6 but were also found in cluster 5 when they also expressed a high leaf area. Finally, cluster 7 was characterized by a LMR under 43%.

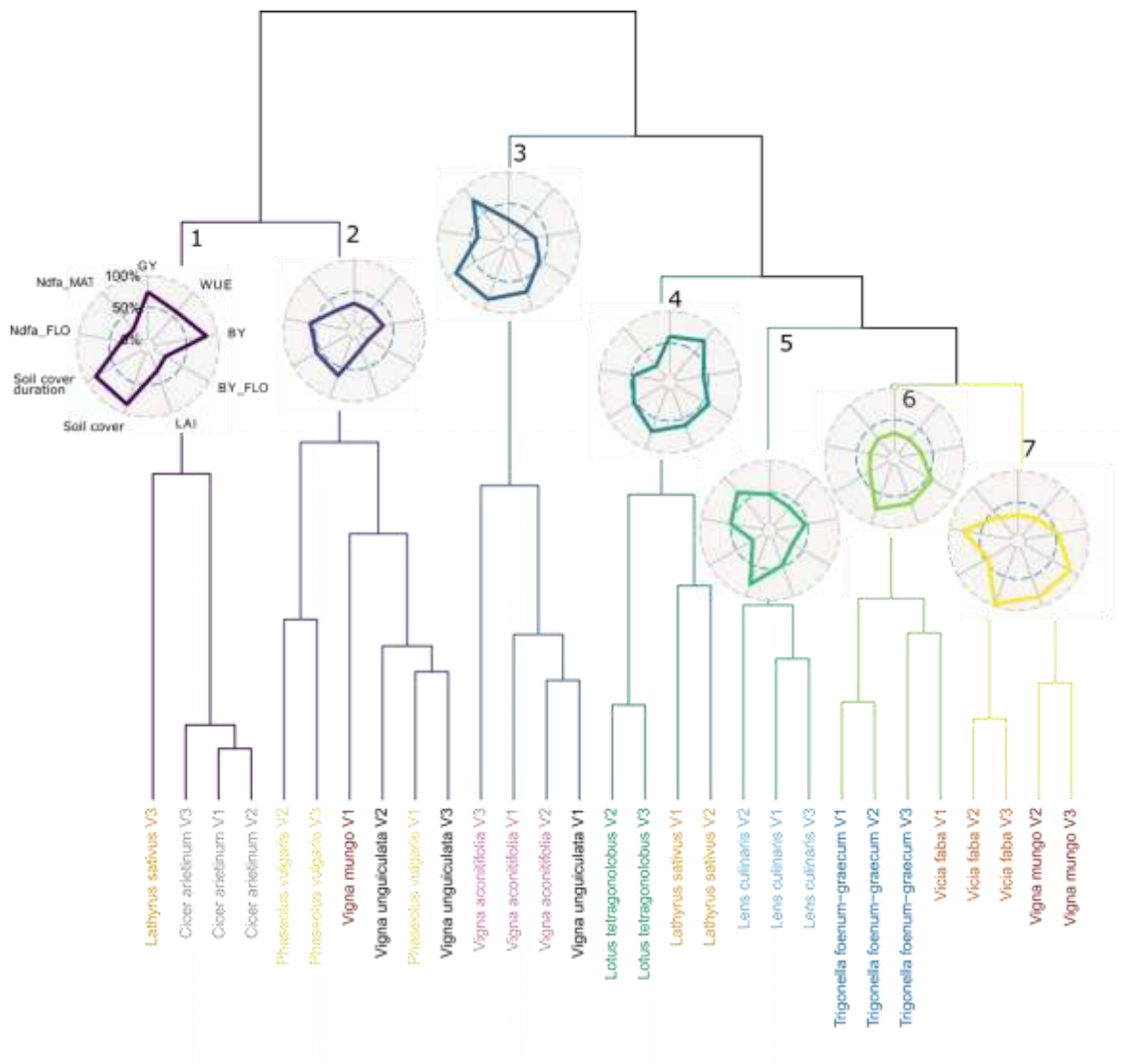


Figure 12 : (a) Hierarchical clustering analysis (HCA) of the 30 varieties based on mean agroecosystem property values ($n = 3$). The names of varieties belonging to the same species are written in the same color. (b) Mean (standardized to maximum) agroecosystem properties of each cluster. Colors correspond to branch color in the HCA.

Relying on mean decrease in Gini impurity, plant N content at maturity was the second most important trait to discriminate the clusters. Phyllochron, SNC, SLA and LL were not selected in the tree and had very low mean decrease in Gini value. LAR was not used in the trees, but had a higher mean decrease in Gini value than LA and LMR. Yet, LA and LMR captured more variability in the PCA than LAR. Finally, cover rate did not appear on the tree despite its high mean decrease in Gini value, probably due to its negative correlation with DDR.

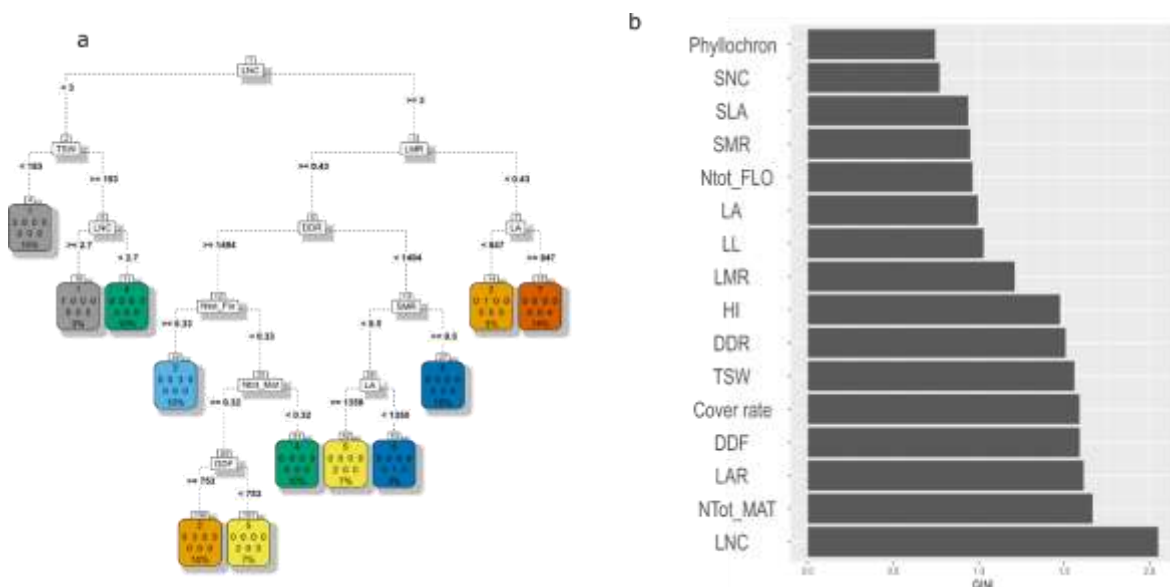


Figure 13 : (a) CART regression for prediction of membership to one of the seven clusters based on agro-ecosystem properties. Root nodes represent single input variables (functional traits) and related split point used to make the prediction. Leaf nodes contain mean, number and percentage of observations of the predicted variable. (b) Gini decrease value for each trait obtained through a random forest procedure.

D. Discussion

I. Trait related to resource acquisition are less stable across growing conditions and varieties than architectural traits

Through functional traits, researchers usually seek to make generalizable predictions across organizational and spatial scales (Adler et al., 2013). Valuable knowledge has been obtained on the functional diversity of managed species from studies that use data collected in various environments and with different managements (Gunton et al., 2011; Yu et al., 2015; Kunstler et al., 2016; Isaac and Martin, 2019; Guiguitant et al., 2020). On the contrary, field measurements sharing similar environment as in the present study deliver a local picture of functional diversity which variations can be solely attributed to genotypic determinants. Several studies have demonstrated that despite the significant plasticity of most plant traits across environments, the classification of species for a given trait remains fairly consistent in space and time (Garnier et al., 2001; Kazakou et al., 2014; Tribouillois et al., 2015). This result contrasts with other studies which have reported a significant

effect of plasticity on species ranking across environments, particularly with respect to traits associated with light acquisition and nitrogen nutrition (Lipowsky et al., 2015; Siebenkäs et al., 2015; Keenan and Niinemets, 2017). These inconsistencies may impair the use of simple aggregation functions (means, medians, etc) for generalized trait-based approaches at the species level.

In the present study, we compared common field measurements to the meta-analysis by Guiguitant et al. (2020) to gain insight into the usefulness of the individual trait-based approach to discriminate combinations of canopy-level aggregated properties viewed as putative agro-ecosystem services. When using the experimental data set, where all data were acquired in a common environment, species rankings on the PC axis summarizing the variation of architectural traits were fairly consistent with literature ranking. However, rankings on the axis summarizing SLA and LNC variation diverged between the two data sets. This contrast mainly concerned warm-season species, particularly *Vigna* sp. for which divergence between the two data sets were also noted in terms of phenology. Number of days required to reach maturity was generally lower in literature than in experimental data. This comparison between local measurements and literature averages suggests some traits are more plastic than others and therefore contribute differently to frame legumes diversity depending on the conditions of observation.

II. Intraspecific variation contributed only marginally to global variability

Results from the PCA showed that the total trait variance was mainly explained by the variance between species. Variation in intraspecific traits is generally considered of marginal importance compared to differences in interspecific traits (McGill et al., 2006; Hulshof and Swenson, 2010). However, plants often exhibit a high intraspecific variation in functional traits, reflecting both heritable genetic variation and phenotypic plasticity. Several recent studies have shown that intraspecific variation in traits accounts for a substantial proportion of overall plant community variability (Jung et al., 2010; Albert et al., 2011; Roscher et al., 2015; Siefert et al., 2015). The small number of varieties considered in this study may have not been able to highlight such diversity. Therefore, our results confirmed variation in intraspecific traits is of marginal importance

III. Leaf morphological traits confirmed as variability drivers among pulses

Guiguitant et al., (2020) found that seed and leaf traits exhibited the greatest variability among 43 pulse species. In accordance with Westoby's LHS (leaf-height-seed) scheme (Westoby, 1998), this was indicative of significant functional structuration. In the present study, leaf traits (LA, LL) remained an important variability driver that was correlated to the first PCA axis, whereas seed size, associated to the first axis in the literature analysis, poorly contributed to the total variance across

varieties (associated only with the fourth PCA axis) and was decoupled from leaf size, which is consistent with LHS scheme.

The experimental data confirmed that leaflet length is an important determinant of legume diversity. This trait was strongly related to LNC and to a lesser extent SLA. This finding can be related to the well known leaf economic spectrum (LES; Wright et al., 2004). The LES reflects a trade-off between a leaf's lifespan and its maximum photosynthetic rate. Alike LNC, the size and organization of leaves appear to be a frequent component of ecological strategies : plants with leaves divided into small leaflets would have a more productive photosynthetic apparatus (Leavitt et al., 1979). Yet, relations between LNC and photosynthesis in grain legume have been questioned (Adams et al., 2016). Thus, the fact that this axis reflects a gradient in leaf photosynthetic capacity is uncertain. However, a high LNC could have other biological implications such as remobilization to the reproductive organs (Davies et al., 2000; Schiltz et al., 2005). Indeed, SNC was correlated with LNC in our experiment. Pulses are also known for their often nitrogen-rich seeds compared to other crop species (Sinclair and Wit, 1975; Hardwick, 1988). Thus, small leaflet concomitantly with a high LNC could be important for reproductive strategy through remobilization.

IV. LMR and LAR reflect diverging biomass investment strategies

Leaves play an especially crucial role in carbon assimilation, water relations and energy balance (Ackerly et al., 2002). Consequently, the biomass allocated to leaves can have a significant effect on plant resource acquisition. Here, investment in leaves (area based) seems tightly linked to nitrogen accumulation in plant and reallocation to seeds. Data showed a negative correlation between LAR and LA. This balance obviously depends on the total biomass produced per plant. Yet, biomass invested in leaves (LMR), which contributed to the total variability of traits via the second axis of the experimental PCA, was not correlated with area-based leaf investment (LA, LAR). In theory, increased LAR provides more leaf area for light capture per unit of biomass (Brenes-Arguedas et al., 2013; Yin et al., 2019). On the other hand, the present experiment highlighted that LMR together with SMR were correlated with traits related to growth rate (cover rate, MAT). Higher biomass allocation to leaves was related to a slow growth. Which is consistent with the third dimension of LSH scheme related to the trade-off between “fast” traits that promote rapid resource acquisition and “slow” traits (Wright et al., 2004; Díaz et al., 2016; Voltaire, 2018) and generally represented by SLA (Poorter and Remkes, 1990; Lambers and Poorter, 1992; Poorter et al., 2009). In the present study, this trade-off seemed to be more related to the percentage of biomass allocated to leaf independently from the leaf area achieved.

V. Yield is inversely related with Ndfa and soil cover

Nitrogen fixation by legumes is generally expected to contribute to improving soil fertility through the mineralization of crop residues such as senescent leaves or stems, or roots. In the current study, Ndfa at flowering and Ndfa at harvest in grain tissue varied almost independently. Clustering revealed three types of “high fixing species”: cluster 7 which appears to have a maximal proportion of N acquired through fixation in plant tissues near flowering, cluster 3 which maximum proportion of N from atmospheric origin was reached after flowering and cluster 5 which seems to maintain a good proportion of fixation in N acquisition from flowering to the end of seed filling. On the other hand, cluster 1 or 6 expressed a low N fixation capacity. “High yielding” species were clustered in cluster 1 and, to a lesser extent, cluster 4 both concerned by low fixation. Cluster 5 fixed a lot of N during flowering and produce a decent yield. However, yield in cluster 5 was lower than that of clusters 1 or 4. Conversely, the very low yielding species (cluster 3) expressed a significant increase in relative Ndfa between flowering and maturity. Other multiple species comparisons confirmed the negative relation between biological nitrogen fixation and seed yield (Liu et al., 2019). Some studies reported biological nitrogen fixation over a certain levels results in a reduction of yield (Mesfin et al., 2020).

The multi-faceted benefits of cover crops are well known (Blanco-Canqui et al., 2015), but land cover is often not considered for cash crops and the interaction between cover capacity and yield is rarely discussed. Maximum cover and cover duration appeared to be related to biomass at flowering and LAI as well as to yield properties. Cluster 7 expressed an important biomass and a large leaf area at flowering which resulted in an important cover. However, cluster 1 and 3 had a low biomass at flowering and a relatively high maximum cover which lasts long. This may be the result of a delayed senescence. Except for cluster 1, most of those species had low yield. Delayed senescence, by prolonging photosynthesis, is usually expected to contribute to increasing the yield of high-carbon crops such as cereals (Gregersen et al., 2013). However, studies suggest that the stay-green trait may have limited value for high N species (Ismail et al., 2000; Kumudini, 2002) as its value lies in an improvement of remobilization capacity. This corroborate the absence of relation we found between yield and duration of ground cover.

VI. Trait-based predictions of agro-ecosystem properties

Understanding the links between functional traits and agro-ecosystem functioning is fundamental for optimal management of agro-ecosystem services (Zhang et al., 2007; Litrico and Violle, 2015; Wood et al., 2015). As noted earlier, leaf characteristics and especially LNC were crucial in describing pulse diversity. Other traits such as LAR, total nitrogen, seed size and cycle length are also important, which is consistent with the study of Guiguitant et al. (2020). The decision tree did not highlight all these traits but discriminated specific functional characteristics for each cluster. Cluster 1 and 4 (high yielding species) were characterized by low LNC values which is consistent with their low N fixation.

Cluster 7 (high biomass at flowering) was characterized by a large leaf area but a low biomass investment in leaves. Clusters 6 and 5 (medium maximum soil cover, and low value for every other properties) were characterized by a short cycle, with cluster 5 (high nitrogen fixation) having a higher leaf area. Clusters 2 (medium value for every properties) and 3 (high Nitrogen fixation at maturity and long cover duration) were characterized by a long cycle and high nitrogen content at maturity or flowering respectively.

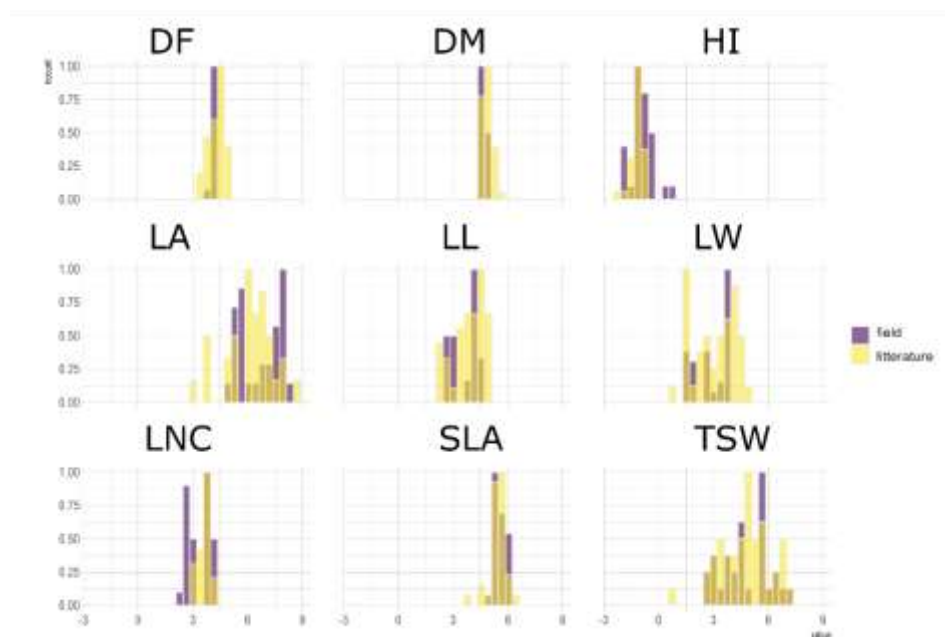
To conclude on the highlighted linkages, a high LNC value does not translate into higher yields but is generally associated with a high N fixation. Investment in leaf area is important for N₂ fixation at flowering but is associated with rapid senescence and low yield. Biomass investment in leaves and a short cycle coincide with low values for almost every property except maximum soil cover. Finally, long cycle and high N content at maturity are related to delayed senescence and maintenance of N₂ fixation along pod filling.

E. Conclusion

Legumes are usually presented as very beneficial for agro-ecosystem through the provision of a large set of services such as human protein nutrition, soil fertility, and weed suppression. Yet those services cannot all be found at the same time in a single legume species. Our experimental results highlighted trade-offs between agroecological properties supported by different legume species. Thanks to this approach we were able to demonstrate the relevance of LNC, LAR, and phenology to predict most of the trade-offs observed for agro-ecosystem properties in those conditions.

Several previous studies demonstrated the usefulness of trait value aggregation at the species-level to implement trait-based approaches for a better knowledge of the plant-agro-ecosystem functioning relationships and increasing our predictive capacity, (Lavorel and Garnier, 2002; Violle et al., 2007; Garnier and Navas, 2012; Kazakou et al., 2014; Martin and Isaac, 2015; Wood et al., 2015; Guiguitant et al., 2020). However, the present study advocates further for a systematic characterization of the functional diversity (and its plasticity), including intra-specific variability. We argue that the trait-based approach may be easier to implement in crop species where varieties origin and genetics are often better described and accessible, compared with the huge genetic variation encountered in populations of spontaneous species. Application of such trait-based approach would rapidly benefit the selection of species/varieties for specific targeted agro-ecosystem services provisioning under specific (environmental or management) conditions.

F. Supplementary materials



Supplementary figure S3 : Comparaision of traits range for data measured in the present field experiment over 30 varieties issued from 10 species of pulses and data collected in literature in Guiguitant et al. 2020 for 43 species of pulses.

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**CHAPTER 4. *AN EXPLORATION OF THE
VARIABILITY OF PHYSIOLOGICAL RESPONSES TO SOIL
DRYING IN RELATION WITH C/N BALANCE ACROSS
THREE SPECIES OF THE UNDER-UTILIZED GENUS
VIGNA GENUS***



Special Issue Article

An exploration of the variability of physiological responses to soil drying in relation with C/N balance across three species of the under-utilized genus *Vigna*

Julie Guiguitant, H el ene Marrou , Denis Vile , Thomas R. Sinclair, Deepti Pradhan, Martha Ramirez, Michel Edmond Ghanem

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ABSTRACT:

The genus *Vigna* (Fabaceae) is an agriculturally important taxon, which includes several crop species such as cowpea (*Vigna unguiculata* L.), mung bean (*Vigna radiata*) and azuki bean (*Vigna angularis*). Most studies have focused on cowpea (*Vigna unguiculata* (L.) as a drought-resistant crop, although insights on the mechanisms that confer this species the ability to grow in dry environment are still not fully resolved. The diversity of this rich genus has been overlooked in many physiological studies. This study explores the physiological mechanisms of response to soil drying (N₂ fixation, transpiration rate and changes in C and N allocation) across three species of the *Vigna* genus: *Vigna radiata*, *Vigna unguiculata*, *Vigna vexillata* (tuber cowpea). A significant variability was found for the threshold in decline of N₂ fixation with soil drying among the studied *Vigna* accessions. Less variability was observed in the transpiration threshold. Through the analysis of leaf traits variation under well-watered and water-deficit conditions, we were able to relate the variability in N₂ fixation and transpiration response to C/N metabolism modifications resulting in different allocation of carbon and nitrogen to leaves under water deficit.

A. Introduction

About one billion people live in arid and semi-arid regions, and 40% of them live on less than a dollar a day. Pulses usually constitute a key component of human diets in these regions (Foyer *et al.*, 2016b). Their ability to assimilate atmospheric N₂ symbiotically with rhizobial bacteria gives those crop species a major role, both agronomically and economically. These crops are often produced under dry conditions. A better understanding of the mechanisms controlling water use, N₂ fixation and productivity of pulses, is likely to result in the development of better-adapted cultivars for different locations and farming systems.

Among pulses, the *Vigna* genus is a significant agricultural taxon, which includes about ten domesticated species (Takahashi *et al.*, 2016). Since *Vigna*'s wild and cultivated species can be found in a wide range of climates, from arid areas to tropical regions (Tomooka *et al.*, 2014), it is expected that they have developed a wide range of drought response strategies. In addition, as they exhibit variable N₂-fixing capacity which may reflect their adaptation to harsh environments where resources are limited, these legumes have a high propensity to be used in low-input agricultural systems (Takahashi *et al.*, 2016).

Genetic variation of drought response strategies have been investigated in some domesticated *Vigna* accessions (Agbicodo *et al.*, 2009; Kumar and Sharma, 2009; Jørgensen *et al.*, 2010; Scotti-Campos *et al.*, 2013). Drought impact on yield has particularly been investigated in cowpeas (*Vigna unguiculata*), one of the most economically important species of this genus (Scotti-Campos *et al.*, 2013). Cowpea is generally considered to be drought tolerant (Agbicodo *et al.*, 2009; Belko *et al.*, 2012) and shows substantial ability to survive and maintain high yield when subject to extreme soil-water deficit during the vegetative phase (Turk, Hall and Asbell, 1980).

In the current study, two physiological traits are studied: early partial stomatal closure with soil drying which is an important trait for soil water conservation (Sinclair, 2017a), and sustained N₂ fixation activity with soil drying, which is critical for growth under water-deficit (Serraj, Sinclair and Purcell, 1999; Aranjuelo, Arrese-Igor and Molero, 2014). Genetic variation in some legume crop species has been identified for these two traits. Soil water content at which transpiration decreases with soil drying has been investigated in lentil (*Lens culinaris* Medikus) (Ghanem *et al.*, 2017), chickpea (*Cicer arietinum* L.) (Zaman-Allah, Jenkinson and Vadez, 2011; Sivasakthi *et al.*, 2017), soybean (*Glycine max* (L.) Merr.) (Hufstetler *et al.*, 2007; Sinclair, 2017b), peanut (*Arachis hypogaea* L.) (Bhatnagar-Mathur *et al.*, 2007; Devi *et al.*, 2009; Devi and Shekoofa, 2017), common bean (*Phaseolus vulgaris* L.) (Devi *et al.*, 2013) ; and cowpea (*Vigna unguiculata*) (Sinclair *et al.*, 2015). Similarly, variation has also been identified for the soil water content at which N₂ fixation activity decreases in soybean (Sinclair *et al.*, 1987, 2000; Sall and Sinclair, 1991), peanut (Devi, Sinclair and Vadez, 2010), common bean (Devi *et al.*, 2013), cowpea (Sinclair *et al.*, 1987, 2015) and black gram (*Vigna mungo*) (Sinclair *et al.*, 1987). Both traits have now been incorporated into elite lines of soybean for release as high-yield germplasm for drought conditions (Devi *et al.*, 2014; Carter and Tegeder, 2016).

In the case of the *Vigna* genus, these two traits have received little attention and only for cowpea was they been considered. The influence of drought on cowpea N₂ fixation activity has been reported at the enzymatic level (Figueiredo, Burity and De França, 1999; Figueiredo *et al.*, 2007), but the impact of such molecular changes have not been investigated at nodule and plant scale in Figueiredo's studies. Sinclair *et al.* (2015) measured *in situ* the N₂ fixation activity at plant scale of ten genotypes and found that the threshold values for the fraction of transpirable soil water (FTSW) causing fixation

decrease varied significantly between accessions, from 0.24 to 0.59. Other studies reported decreases in plant N₂ fixation activity with soil drying as compared with leaf capacity for CO₂ assimilation (Pararajasingham and Knievel, 1990; Sinclair and Serraj, 1995; Silveira *et al.*, 2003). In each case, N₂ fixation rates declined more rapidly than C accumulation capacity indicating a sensitivity of N₂ fixation to soil drying. Sinclair *et al.* (2015) reported no correlation between the thresholds for decline in transpiration and N₂ fixation rates in cowpea and concluded that among the studied grain legumes, the N₂ fixation rate of cowpea appears to be most tolerant to soil drying (Sinclair *et al.*, 2015). However, lack of genetic and phenotypic information about other *Vigna* species with respect to these traits limits conclusions about the opportunities for abiotic stress-tolerance improvement (Iseki *et al.*, 2018).

Determinants of the genotypic variability of the responses of fixation and transpiration to drought could rely on differential modifications of the plant C/N balance. Some studies indicate that N₂ fixing activity regulation could be linked to C/N equilibrium, allowing to optimize plant nitrogen requirements at a minimal carbon cost. Understanding the regulation of C/N balance under drought implies exploring the source-sink relationships. Ecologists have proposed an approach that relates plant functional traits to plant strategies for resource acquisition (Grime *et al.*, 1997b; Grime, 2009b; E Garnier and Navas, 2012b). According to this approach, species can be classified along a gradient of resource use strategies, from the "acquisitive" ones to the "conservative" ones. Specific leaf area (SLA) and the foliar nitrogen content (LNC) seem to be effective indicators to capture the gradient from "acquisitive" species (high SLA and LNC) to "conservative" ones (low SLA and LNC) (Wright *et al.*, 2004b). However, only few studies have truly investigated the relationships between these traits and plant physiological functioning under water-stress conditions, especially when it comes to regulation of nitrogen fixation under water deficit.

To study the genetic variation of responses to soil water deficit within the genus *Vigna*, nine representative accessions of three *Vigna* species were selected to represent the three most important subgenera from an agronomic and geographic point of view (Takahashi *et al.*, 2016): cowpea, *Vigna unguiculata* (African *Vigna* Cultigens; $n = 4$), mung bean, *Vigna radiata* (Asian *Vigna* Cultigens, $n = 3$) and *Vigna vexillata*, tuber cowpea (Eurasian *Vigna* Cultigens, $n = 2$). The sensitivity of transpiration rate and symbiotic N₂ fixation activity to progressive soil drying were first measured. Then, the relationships between the timing of both responses to water deficit and key leaf traits (SLA and LNC) were measured.

B. Material and methods

I. Plant material and growing conditions

Seeds were obtained from the U.S. National Plant Germplasm System (Plant Genetic Resources Conservation Unit, Griffin GA, USA). We selected a total of nine accessions : four accessions of cowpea (*Vigna unguiculata*) ; two accessions of tuber cowpea, a lesser known but potentially important food legume coming from the Eurasian *Vigna* Cultigens (subgenus *Plectotropis*) (Karuniawan et al. 2006); and three accessions of mung bean belonging to the subgenus *Ceratotropis*, also known as the Asian *Vigna*. This sub-genus was previously treated as part of the *Phaseolus* genus until 1970 (Verdcourt, 1970).

Due to the limited capacity of the N₂-fixing measurement system, which was limited to 40 individual plants, two sequential experiments were conducted to obtain data for all nine accessions ($n = 8$). On 2 May and 10 June 2019, three seeds were sown in 2.2-L individual pots (10-cm diameter, 30-cm tall) constructed from polyvinyl chloride pipes, and filled with sandy-clay soil (69% sand, 18% silt and 13% clay). The soil was inoculated with a commercial inoculant containing *Bradyrhizobium* (Guard'n Seed Inoculant), which resulted in nodulation of all plants. After seedling emergence, each pot was thinned to one plant. All pots were kept well-watered until the dry-down experiments were initiated, four weeks after emergence. The plants were grown in a greenhouse located in Raleigh, NC (46°35' N, 39°78'W). The temperature in the greenhouse was set at 28 °C day and 23 °C night. No supplemental lighting was used to increase the length of the day, and if an occasional flower emerged, it was detached from the plant.

II. Dry-down experiment and N fixation activity

The protocol for determination of N₂ fixation activity during the soil- drying cycle was similar to that originally presented by Sall and Sinclair (1991) and described in detail by Devi and Sinclair (2013). When plants were approximately 4 weeks (17 may and 10 june), pots were tightly sealed so that the rhizosphere could be exposed to the flow of an acetylene-air mixture (1:9 by volume) during the measurement of nodule activity. The evening before the experiment began, all the pots were watered until they dripped, and once the pots stopped dripping, they were weighed to obtain the initial weight. Afterwards, all the pots were weighed each day at the same hour. The eight pots of each genotype were divided into three well-watered pots and five drying pots. Water was added daily, as needed. Well-watered pots were maintained at 100 g under their initial weight to avoid dripping. Pots under water deficit were also irrigated if needed so that their net daily water loss did not exceed about 70 g.

Each morning, air was initially flowed through the sealed pots for at least half an hour. This was followed by flowing the acetylene–air mixture through the pots. Acetylene is preferentially reduced by the enzyme nitrogenase in nodules. Stable levels of ethylene in the pot outflow were obtained in less than 15 min of exposure to the acetylene-air mixture, then three gas samples were rapidly taken with 1-mL syringes from the outflow from each pot. After collection of the gas samples, the pots were flushed with air for a further 60 minutes. This system allowed a very short (< 20 min) daily exposure to acetylene and therefore avoided disturbing the plants. The gas samples were analyzed for ethylene concentration using a gas chromatograph with a flame ionization detector. The protocol was repeated daily for 15 and 19 days (until the plants plant NTR fell below 0.1)

At harvest, all leaves were detached from each plant to determine total leaf area. Individual leaves were placed in an oven at 65 °C during 3 days to determine leaf dry weight. Leaf N content was determined on dried samples with the Flash EA 1112 elemental analyzer (Thermo Scientific, Waltham, MA), based on the Dumas method described by Watson and Galliher (2001). Samples were combusted at a temperature of 1800 °C with pure oxygen. The nitrogen oxides produced were reduced to nitrogen, separated from other byproducts by gas chromatography and measured using a thermal conductivity detector. The percentage of nitrogen was calculated with Eager smart software (Thermo Scientific, Waltham, MA). Specific leaf area (SLA, $\text{cm}^2 \text{g}^{-1}$) and leaf N content (LNC, mg N g^{-1}) were calculated for each plant.

III. Data analysis

The time-course of both transpiration rate (TR) and acetylene reduction activity (ARA) was analyzed using of a double normalization procedure. The first step was a computation on each day the ratios of TR and of ARA for each plant grown under water deficit by dividing each value by the daily average of TR and ARA of the three plants grown under well-watered conditions. This ensured that daily trait variation due to changes in the greenhouse conditions was minimized. The second normalization was done to allow data comparison among the five drying pots of each accession without having to rely on leaf area measurements. The daily relative transpiration rate – or relative acetylene reduction activity – was divided by its initial average (computed on the first few days of experimentation before any reduction of transpiration) to obtain normalized transpiration rate (NTR) and normalized acetylene reduction activity (NARA). Therefore, NTR and NARA values of each plant subjected to soil drying were initially centered on 1.0.

NTR and NARA values were plotted against the fraction of transpirable soil water (FTSW). FTSW was determined on a daily basis for each pot as the difference between the current daily and final weight divided by the difference between the initial and final weight. The final weight of the pot was defined as occurring when the NTR fell below 0.1.

A piecewise linear regression with two segments for NTR and NARA was done for each genotype by combining in the regression the individual data for each of the five drying plants. The regression was done using the “segmented” R package (Muggeo, 2008). Equation 4 gives the equation of the regression model for NTR fitted on normalized transpiration data.

$$\begin{cases} NTR_i = \beta_0 + \beta_1 FTSW_i & \text{if } FTSW_i < BP \\ NTR_i = \beta_2 FTSW_i + \beta_0 + (\beta_1 - \beta_2) * BP & \text{if } FTSW_i > BP \end{cases} \quad (\text{Equation 5})$$

In equation 1, β_0 is the intercept of the first segment, β_1 is the slope of the first segment, β_2 is the slope of the second segment, and BP is the FTSW threshold between the two linear segments. The segmented R package uses the Davies’ test to check whether there is a significant difference in slope before and after the breakpoint. The alpha value of this test was considered to be 0.05.

For each accession, the data were first tested with the Davies test to see whether or not there was a threshold, and then, depending on the result, data were described with either a two-segment regression or a linear regression. A simple Wald’s test (McCullagh and Nelder, 1989) was used to test if the initial slope before the soil dried to the BP was significantly different from 0 for each accession. Although the test showed that for some accessions, this slope was significantly different from 0, we chose to set it to 0 for all accessions, both for NTR and NARA dynamics, as it was done in other studies. Indeed, small variations found for this slope, measured at the beginning of the experiments, were considered essentially due to possible damages provoked by the sealing or leakage in the system. Comparison of thresholds between accessions was based on the 95% confidence interval for this variable.

C. Results

I. NARA and NTR variation along FTSW gradient

NTR was essentially steady until FTSW fell below a particular threshold FTSW and then decreased linearly thereafter (Figure 14a). The two-segment linear regression model described well the response pattern of NTR and explained more than 85% of variation in all accessions (Table 6). The FTSW threshold values for transpiration decline ranged from 0.23 ± 0.03 (\pm CI) for *V. radiata* accession PI406387 and *V. vexillata* accession GRIF12425 to 0.33 ± 0.04 for a *V. unguiculata* accession GRIF14490 (Figure 14a). The lowest thresholds were significantly different from the highest ones as their 95% confident intervals did not overlap.

The response of NARA to soil drying was also described by two-segment linear regression model (Figure 14b). However, the data were much more scattered for NARA than for NTR. The coefficient of determination (R^2) ranged from 0.30 to 0.70 among accessions (Table 6). The wide range of NARA threshold values illustrated clear differences among accessions (Figure 14). Threshold increased from very low values for a group of three accessions (PI656385 and GRIF14490 and GRIF12354)

exhibiting a breakpoint at 0.08 ± 0.04 (\pm max CI) to intermediate values for accessions PI293520, GRIF12425 and PI406387 (Figure 14b). These two groups significantly differed from a third group for which N₂ fixation started to decrease earlier in soil drying with FTSW breakpoint values ranging from 0.37 ± 0.12 (PI164301) to 0.42 ± 0.09 (PI406400). Thresholds significantly differed between accessions within species but not among species (Table 6).

Table 6 : Fraction of transpirable soil water (FTSW) at the breakpoint for the decrease in normalized acetylene reduction activity (NARA) and normalized transpiration rate (NTR) with soil drying of 9 *Vigna* accessions. Associated with the slope β_1 of the following decrease. Breakpoints and slopes are followed by different letters based on the 95% confidence interval. the initial phyllochron is expressed as the number of cumulative growing degrees days (GDD) it take for a new leaf to grow. Letters are based on the analysis of covariance. This table also contain the NARA value when FTSW is equal to the breakpoint and when FTSW is equal to 0.

Accession	species	NTR vs FTSW			NARA vs FTSW		
		BP \pm CI	$\beta_1 \pm$ CI	R ²	BP \pm CI	$\beta_1 \pm$ CI	R ²
PI634767	<i>V. unguiculata</i>	0.25 ± 0.03 bc	3.63 ± 0.6 ab	0,94	0.39 ± 0.31 abc	1.40 ± 1.4 b	0.29
PI656385	<i>V. unguiculata</i>	0.30 ± 0.03 ab	2.99 ± 0.4 b	0,93	0.08 ± 0.03 c	9.41 ± 4.0 a	0.60
GRIF14490	<i>V. unguiculata</i>	0.33 ± 0.04 a	2.70 ± 0.4 b	0,93	0.08 ± 0.04 c	9.85 ± 6.8 a	0.45
PI293520	<i>V. unguiculata</i>	0.26 ± 0.04 abc	3.30 ± 0.6 ab	0,88	0.1 ± 0.05 bc	6.44 ± 4.5 ab	0.33
GRIF12354	<i>V. radiata</i>	0.27 ± 0.04 abc	3.16 ± 0.6 ab	0,86	0.08 ± 0.04 c	6.31 ± 5.1 ab	0.21
GRIF12425	<i>V. radiata</i>	0.23 ± 0.02 c	3.90 ± 0.5 a	0,93	0.11 ± 0.03 bc	6.09 ± 2.2 a	0.52
PI164301	<i>V. radiata</i>	0.25 ± 0.05 bc	3.71 ± 0.9 ab	0,85	0.37 ± 0.12 a	1.94 ± 0.8 b	0.68
PI406387	<i>V. vexillata</i>	0.23 ± 0.03 c	4.05 ± 0.7 a	0,94	0.17 ± 0.05 b	4.22 ± 1.7 ab	0.75
PI406400	<i>V. vexillata</i>	0.31 ± 0.04 ab	3.04 ± 0.5 ab	0,87	0.42 ± 0.09 a	2.04 ± 0.6 b	0.65

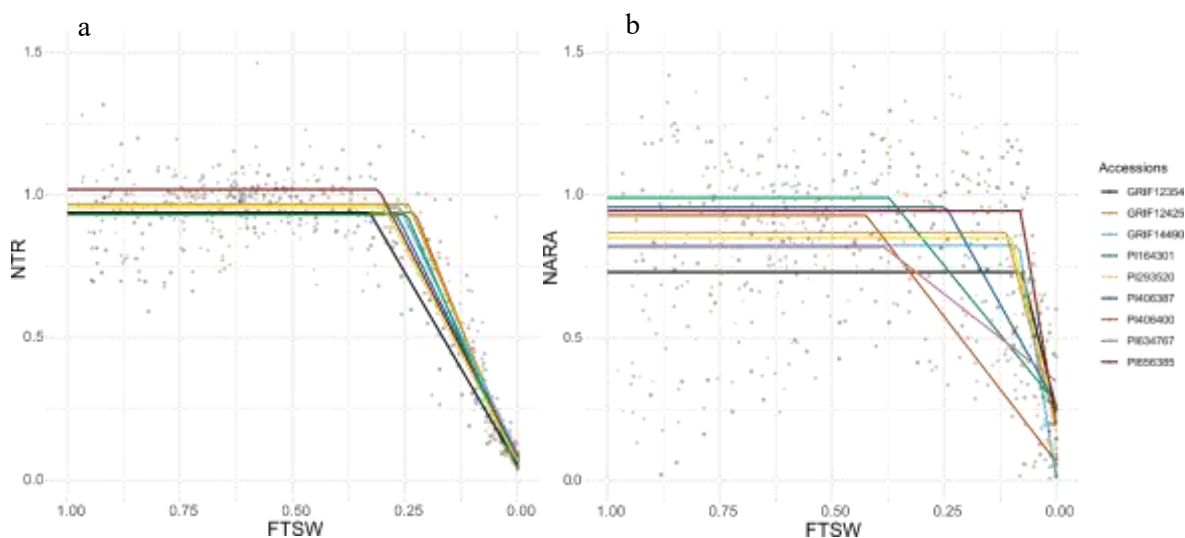


Figure 14 : Plots for all 9 *Vigna* accessions of (a) normalized transpiration rate (NTR) vs. fraction of transpirable soil water (FTSW) and (b) normalized acetylene reduction activity (NARA) vs. fraction of transpirable soil water (FTSW). line result of each accession segmented regression (see Table 6) and the existence of a significant breakpoint was tested through a Davis test

11. Variation of leaf traits

SLA values (Table 7) measured at the end of the experiment varied significantly among accessions ($P < 0.001$). Values for well-watered plants ranged from $155.3 \pm 64.4 \text{ cm}^2 \text{ g}^{-1}$ (PI164301) to $318.4 \pm 89.7 \text{ cm}^2 \text{ g}^{-1}$ (GRIF12425), and from $220.9 \pm 30.3 \text{ cm}^2 \text{ g}^{-1}$ (PI164301) to $378.5 \pm 19.5 \text{ cm}^2 \text{ g}^{-1}$ (GRIF14490) for plants under water deficit. Well-watered plants had lower value of SLA than plants grown under water deficit with the only exception of GRIF12425 which had a higher SLA under well-watered conditions. This resulted in a significant difference between stressed and unstressed SLA ($P < 0.001$). The SLA under water deficit was $20.5 \pm 7.4 \%$ to $66.6 \pm 6.1 \%$ higher than SLA under well-watered conditions. The percentage of reduction of SLA between well-watered and stressed plants observed for GRIF12425 was equal to $26.6 \pm 8.5 \%$.

Table 7 : average Specific leaf Area (SLA; $\text{cm}^2.\text{g}^{-1}$), leaf nitrogen content (LNC; mg N g^{-1}) standard deviation (SD) per accessions. Traits were measured at the end of the dry down experiment on five water-stressed (WS) plants and three well-watered plants (WW). SLA and LNC increase are the difference between WW and WS plant express as a percentage of the WW value. Values followed by different letters are significantly different based of the analysis of variance.

Accession	Species	treatment	SLA \pm SD ($\text{cm}^2.\text{g}^{-1}$)	SLA increase %	LNC \pm SD (mg N g ⁻¹)	LNC increase %
GRIF12354	<i>V. radiata</i>	WS	244.5 ± 21.1 abf	39.8 ± 12.1 ac	30.2 ± 2.2 ad	56.5 ± 11.5 ab
		WW	174.9 ± 34.2 ab		19.3 ± 10.2 a	
GRIF12425	<i>V. radiata</i>	WS	233.8 ± 27.2 abd	-26.6 ± 8.5 d	25.4 ± 3.1 abc	0.4 ± 12.5 d
		WW	318.4 ± 89.7 cdhf		25.3 ± 19.7 ab	
GRIF14490	<i>V. unguiculata</i>	WS	378.5 ± 19.5 h	52.4 ± 7.9 ac	37.8 ± 3.5 cd	59.7 ± 15.1 ab
		WW	248.3 ± 63.7 abf		23.6 ± 4.5 abc	
PI164301	<i>V. radiata</i>	WS	220.9 ± 30.3 abd	42.2 ± 19.5 ac	24.6 ± 4.0 abc	36.7 ± 22.4 bd
		WW	155.3 ± 64.4 a		18.0 ± 5.2 a	
PI293520	<i>V. unguiculata</i>	WS	273.2 ± 16.9 abfg	20.5 ± 7.4 c	36.2 ± 3.8 bd	78 ± 18.9 a
		WW	226.6 ± 49.5 abce		20.3 ± 1.5 ab	
PI406387	<i>V. vexillata</i>	WS	345.8 ± 50.7 fh	53.7 ± 22.5 ab	4.21 ± 3.6 d	87.5 ± 16.3 a
		WW	224.9 ± 12.2 abf		22.0 ± 2.8 abc	
PI406400	<i>V. vexillata</i>	WS	366.6 ± 73.3 gh	23.6 ± 24.7 dc	30.4 ± 3.9 ad	3.1 ± 13.4 cd
		WW	296.5 ± 75.3 bh		29.5 ± 9.2 ad	
PI634767	<i>V. unguiculata</i>	WS	313.5 ± 11.5 defh	66.6 ± 6.1 a	30.7 ± 4.9 ad	46.4 ± 23.1 abc
		WW	188.2 ± 3.7 abc		21.0 ± 5.7 abc	
PI656385	<i>V. unguiculata</i>	WS	333.4 ± 33.0 efh	49.3 ± 14.7 ab	32.0 ± 7.6 ad	50 ± 35.5 ab
		WW	223.3 ± 33.9 abce		21.3 ± 4.0 ab	

LNC values ranged from $18.0 \pm 5.2 \text{ mg N g}^{-1}$ (PII64301) to $29.5 \pm 9.2 \text{ mg N g}^{-1}$ (PI406400) under well-watered conditions and from $24.6 \pm 4.0 \text{ mg N g}^{-1}$ (PII64301) to $41.2 \pm 3.6 \text{ mg N g}^{-1}$ (PI406387) under water deficit. LNC was significantly increased under water deficit ($P < 0.001$). LNC values under water deficit were $0.4 \pm 12.5\%$ (GRIF12425) to $87.5 \pm 16.3\%$ (PI406387) higher than LNC values under well-watered condition. This percentage increase was correlated with the percentage increase observed for SLA ($r = 0.63$).

LNC was positively correlated with SLA both under well-watered ($r = 0.89$; $p\text{-value} < 0.01$) and water deficit conditions ($r = 0.67$; $p\text{-value} < 0.05$) and species of *Vigna* were not significantly different from one another under well-watered conditions. *Vigna radiata* had the lower LNC and SLA values except for GRIF12425.

III. Relation between leaf traits and parameters of transpiration and N fixation dynamics

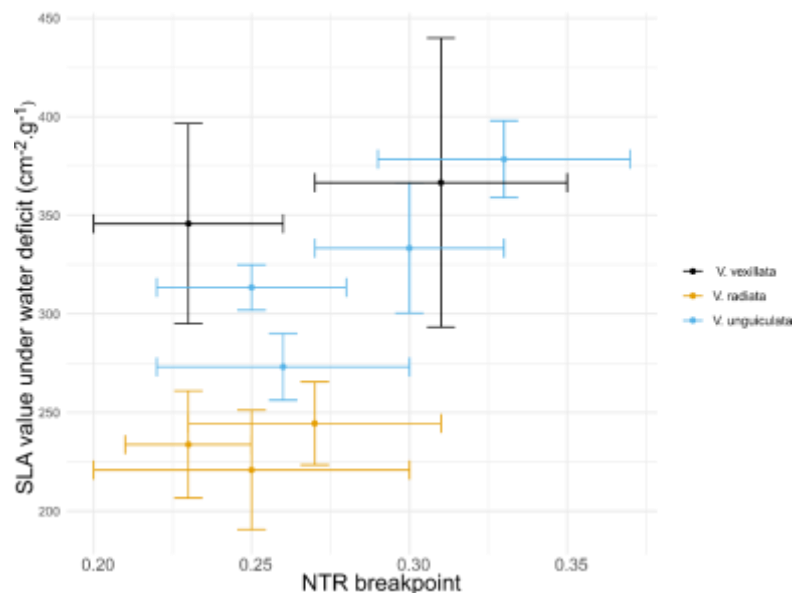


Figure 15 : Correlation between the FTSW breakpoint for the decrease in normalized transpiration (NTR) and the final SLA value measured on plant submitted to water deficit. Horizontal bar represents the Confidence interval à 95% for the breakpoint value and vertical bar represent the standard deviation for the SLA measurements. Colors represent the 3 species studied.

SLA and LNC values under well-watered conditions did not correlate with the thresholds of transpiration and N₂ fixation in response to soil drying. Yet, SLA measured under water deficit was positively correlated to NTR threshold ($r = 0.63$; $P < 0.1$). *Vigna radiata* accessions expressed the lowest SLA values under water deficit concomitantly with a low threshold while *Vigna unguiculata* and *Vigna vexillata* accessions had intermediate to high values of SLA under water deficit and covered the whole range of threshold values (Figure 15).

On the other hand, NARA threshold explained a large part of the difference observed in the variations of LNC between well-watered and water deficit conditions although P-value was only marginally significant ($r = -0.59$; $P < 0.10$). There were two distinct groups (Figure 16); high thresholds (above 0.3) with a small increase in LNC (under 50% increase) and very low thresholds concomitantly with high increase in LNC. This correlation was found more significant if we removed PI634767 for which NARA breakpoint estimation error was substantial. In all cases, correlations were improved by removing GRIF12425, which was the only accession expressing a decrease in SLA under drought.

The NARA threshold did not correlate with the NTR threshold. Three accessions expressed a decrease in NARA prior to the observed decrease in transpiration (*V. unguiculata* PI634767; *V. radiata* PII64301; *V. Vexillata* PI406400). In the remaining accessions transpiration decreased at a higher FTSW than N₂ fixation.

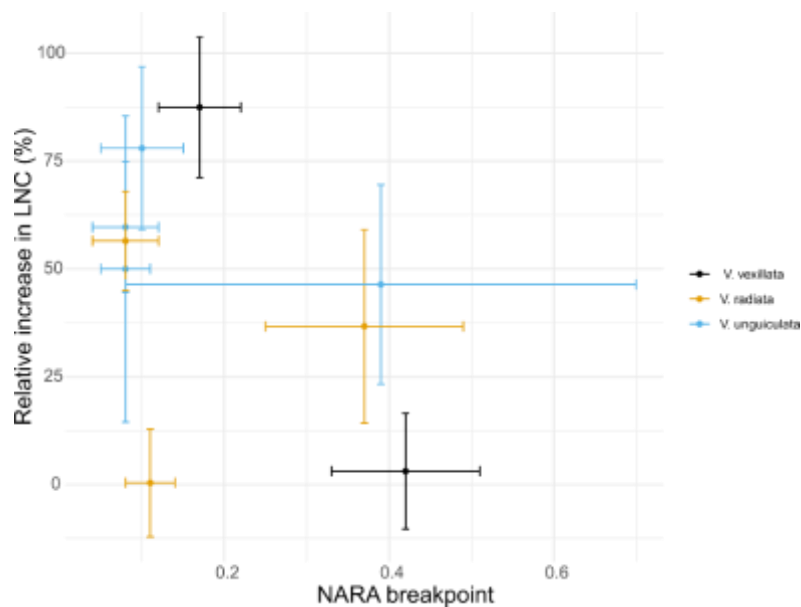


Figure 16 : Correlation between the FTSW breakpoint for the decrease in normalized acetylene reduction activity (NARA) and the relative increase in LNC between well-watered plants and plant submitted to water deficit. Horizontal and vertical bars represents the Confidence interval à 95% for the breakpoint values. Colors represent the 3 species studied.

D. Discussion

I. A very late response of transpiration to soil drying for all *Vigna* sp.

In this study, the FTSW threshold below which transpiration rate declines was measured for different *Vigna* accessions. This initiation of the decrease in transpiration rate is usually interpreted as the result of a stomatal regulation associated with decreasing soil water availability (Ray and Sinclair, 1997). Previous studies on cowpea (*Vigna unguiculata*) have reported threshold values ranging from 0.2 to 0.6 (Sinclair *et al.*, 2015). Our observations on *Vigna* sp. fall within this range (0.23 to 0.33) but are less variable than expected and showed little difference in the response of transpiration to soil drying among species despite their distinct geographical origin. In addition, compared to other species, the FTSW thresholds observed in this study were quite low. Apart from soybean which expresses thresholds between 0.2 and 0.3 (Hufstetler *et al.*, 2007; Sadok *et al.*, 2012); all other legume species (chickpea, lentil, peanut and common bean) had been reported to have thresholds above 0.3 (Bhatnagar-Mathur *et al.*, 2007; Devi *et al.*, 2009, 2013; Zaman-Allah, Jenkinson and Vadez, 2011; Ghanem *et al.*, 2017). Thus, *Vigna*'s transpiration seems less sensitive to soil drying than other legume species.

The main expected benefit of early transpiration limitation with soil drying is to restrict water losses during the crop cycle so that severe late-season drought may be avoided. Increasing availability of water for the crop at the end of the season enhances the chances of sustained crop physiological activity, and hence, to decrease the impact of water deficit on reproductive growth. However, the relative advantage or disadvantage of early stomatal closure in the field might depend on the duration of drought and its timing. Reduced stomatal conductance is also a main limitation to photosynthesis under drought conditions in cowpeas (Medrano, 2002; Singh and Raja Reddy, 2011; Carvalho *et al.*, 2019). Yacine (PI 634767) has been shown to be adapted to rainfed conditions in the dry regions of Senegal (Cissé, Ndiaye and Sène, 2005). This accession was found in this test to have one of the lowest measured thresholds for transpiration reduction amongst the accessions that we have tested. Based on the set of accessions in this test, there seem to be little variability of this trait across the *Vigna* genus. Our study showed that *Vigna* species are very insensitive to soil drying compared to other species. Thus all accessions tested could be characterized as “water-spenders” with a delayed response of transpiration to soil drying, which would confer a drought tolerance to them when subjected to transient drought events.

Further information on how those accessions perform in the field are still needed to draw a solid conclusion on the relation between drought tolerance and transpiration threshold; especially considering that drought tolerance could be conferred through many other traits since several factors and mechanisms (in shoots and roots) operate to enable plants to cope with water deficit.

II. A high tolerance of N₂ fixation to soil drying in *Vigna* sp.

Some of the experimented accessions exhibited N₂ fixation remarkably tolerant to soil water deficit with five of the accessions out of nine having NARA threshold for FTSW below 0.11. This result confirms previous work on cowpea (Sinclair *et al.*, 2015), but contrasts with previous findings on other grain legumes. In previous studies, common bean genotypes had a NARA threshold above FTSW = 0.21 (Devi *et al.*, 2013); in peanut the threshold was not less than FTSW = 0.28 (Devi, Sinclair and Vadez, 2010). In a screen of a large set of soybean genotypes, Sinclair *et al.*, (2000) found only two genotypes with NARA thresholds as low as FTSW = 0.14.

Studies on soybean have identified N₂ fixation as the mechanism most sensitive to water deficit, as it decreases before plant growth and gas exchange (Serraj, Sinclair and Purcell, 1999). This statement doesn't seem to be always true, as other studies have indicated that the opposite may be also true (Serraj and Sinclair, 1998; Devi and Sinclair, 2013). In our study, only three accessions had N₂ fixation more sensitive to soil water deficit than transpiration among which PI634767 showed a substantial error for its breakpoint estimation. In addition, the sensitivity of N₂ fixation to soil drying seemed to be much more variable than the sensitivity of transpiration to soil drying.

Several hypotheses coexist on the mechanisms that could trigger N₂ fixation decline under water deficit: oxygen limitation, reduction in carbon pool, or regulation by N metabolism. Lack of oxygen in response to changes in nodule permeability is generally viewed as the consequence, not the cause, of drought-induced inhibition of nodule metabolism (del Castillo and Layzell, 1995). Although carbon scarcity has been examined in numerous studies (Huang, Boyer and Vanderhoef, 1975; Serraj, Sinclair and Purcell, 1999), results show that not only is carbon not deficient in nodules under drought stress, but that it tends to accumulate (Schubert *et al.*, 1995; Streeter, 2003). The absence of a clear relation between carbon production and N₂ fixation is consistent with the absence of relation found between transpiration response and N₂ fixation response in this current study. As an alternative hypothesis, it has been suggested that plant N status acts as a feedback signal which regulates N₂ fixation (Thavarajah and Ball, 2006; Gil-Quintana *et al.*, 2013). Yet, in our study high NARA threshold are associated with small increase in LNC which doesn't support the hypothesis that nodule activity is retro-regulated by the accumulation of N in leaves. Therefore, it seems that accumulation of N in leaves is a consequence of the maintenance N₂ fixation over a longer period.

III. Leaf traits as indicators of C and N metabolism changes under drought

Drought impact on SLA differed among the 9 accessions tested. We observed an increase in SLA under drought for most accessions except for GRIF12425. In addition, Yacine (PI 634767) – defined earlier as potentially adapted to drought – showed the highest increase in SLA under water-deficit stress. Usually, smaller SLA are regarded as adaptations to drought stress (Abrams, 1994; Pita and

Pardos, 2001), and SLA was found decrease under drought (Liu and Stützel, 2004; Jongrunklang *et al.*, 2008; Painawadee *et al.*, 2009). Increase in SLA under drought could result from changes in carbon allocation in the plant. Studies have indicated that drought could induce a reduction of carbon allocation to leaves possibly related to their strategy to allocate resources to belowground parts (Wellstein *et al.*, 2017). Increasing SLA would allow the plant to compensate lower carbon allocation to leaves, while maintaining a given leaf area (Wang, Hawkins and Letchford, 1998; Palmroth *et al.*, 1999; Wellstein *et al.*, 2017). Moreover, in our study, SLA values under water deficit were correlated to the FTSW threshold of transpiration. This corroborates that early stomatal closure could have impacted carbon gain and modified the allocation to leaves sooner in accessions expressing relatively high breakpoint. Therefore, the increase in SLA observed for most *Vigna* species might be explained by a reduced carbon allocation to leaves.

In this study, a higher N concentration in leaves was observed for all accessions when plants were submitted to soil water deficit except for GRIF 12354 and PI406400 for which there was no significant change. In addition, SLA and LNC values were well correlated in our study. This accumulation of N in leaves under drought was already observed in cowpea in tandem with decrease in C stock which could be explained by a metabolism regulation to balance the production of N and C containing metabolites (Goufo *et al.*, 2017). Increased LNC under water deficit would also be coherent with N₂ fixation being less sensitive to soil drying than transpiration for most accessions. However, this cannot be verified in all accessions.

Finally, our findings were consistent with a classification of *Vigna* species as “acquisitive species” based both on their leaf traits and dynamic responses of transpiration and N₂ fixation to soil drying. High SLA and high LNC are known, in ecology, to lead to an opportunistic, water-spender strategy (Wright *et al.*, 2004), that is consistent with the general behavior of *Vigna* identified in this study. This strategy may be useful in dry, nutrient-poor habitats with extreme temporal fluctuations in resource availability. Rapid resource acquisition and consumption during short resource pulses may effectively pre-empt resource utilization by slower-growth species (Querejeta *et al.*, 2018).

E. Conclusion

Overall, a significant NARA variability in the FTSW threshold was observed among the limited number of genotypes of the three studied species. There was less variability among genotypes in the transpiration threshold. Moreover, intraspecific variability does not seem lower than interspecific variability, emphasizing the interest in comparing multiple species to understand the diversity of the entire genus. The behavior over the carbon allocation to leaves under water deficit and its relation with both N₂ fixation and transpiration response is also intriguing and seems to differ among accessions. Even if this study could not fully decipher what triggers N₂ fixation decline under water deficit and the stress induced sequence of reactions resulting from it, it allowed insight in regards to

several hypotheses on the relations between leaf traits, and soil drying-responses of growth, transpiration, and N₂ fixation. Water-deficit stress affected the C/N balance in the plant differently depending on the plant water conservation strategy and its sensitivity of N₂ fixation to soil drying. *Vigna* genus exhibited interesting performances of N₂ fixation decline only at very low soil water content, and strong de-correlation between transpiration and N₂ fixation thresholds under water-deficit stress.

F. Additional results: *C. arietinum*

I. Introduction

The FTSW threshold at which chickpea transpiration decreases in response to soil drying has already been measured on a few genotypes (Zaman-Allah, Jenkinson and Vadez, 2011; Sivasakthi *et al.*, 2017), yet no information on the regulation of chickpea nitrogen fixation is available in the literature, to date. Most of previous studies measuring the reduction of nitrogen fixation under progressive soil drying focused on warm season species: soybean (Sinclair *et al.*, 1987, 2000; Sall and Sinclair, 1991), peanut (Devi, Sinclair and Vadez, 2010), common bean (Devi *et al.*, 2013), cowpea (Sinclair *et al.*, 1987, 2015) and black gram (*Vigna mungo*) (Sinclair *et al.*, 1987). We hypothesized that, as a cold season pulse, chickpea behavior under soil drying may differ from *Vigna sp.* and species already tested.

II. Material and Methods

The same experiment was performed on seven accessions of *C. arietinum* (chickpea) (see article on *Vigna* for detailed Material and Methods). Seven accessions (provided by USDA, Pullman, WS) were tested in two experimental batches. The experimental procedure and data analysis was similar to the one described for *Vigna sp.* except for the genetic materials and the temperature applied in the greenhouse (22°C day/15°C night). Results obtained for chickpea were compared with those obtained with *Vigna sp.*

Those results were not included in the previous article because those data were of lower quality and required additional treatment steps while time was lacking for that. Data were more scattered because absolute ARA values were very small which increased the error during the normalization procedure. This issue was avoided through an adjustment of the second step of normalization. The normalization was compute using more than the first few days' average allowing to smooth the first steady state. Moreover, a lot of pots had to be removed from the analysis because they were not properly nodulated and thus didn't gave satisfactory result for ARA measurement. With fewer repetitions, data were difficult to published but results were good enough to give insight on the *C. arietinum* response to soil drying.

III. Results

1. *NARA and NTR variation along FTSW gradient*

As for *Vigna sp.*, NTR was essentially steady until FTSW fell below a particular threshold FTSW and decreased linearly thereafter (Figure 17a). The two-segment linear regression model described well the response pattern of NTR to soil drying and explained more than 85% of variation in all accessions (Table 8). The FTSW threshold values for transpiration decline were not significantly different among accessions and averaged at 0.54 ± 0.03 ($\pm 95\%$ CI).

The response of NARA to soil drying was also described by a two-segment linear regression model (Figure 17). However, the data were much more scattered than for NTR. The coefficient of determination (R^2) ranged from 0.14 to 0.96 depending on the accessions (Table 8). The wide range of NARA threshold values illustrated clear differences between accessions (Figure 17). CA04 exhibited the lowest threshold at 0.12 ± 0.15 (\pm max CI) closely followed by Myles and Billy (0.35 ± 0.23 and 0.31 ± 0.09 respectively). Orion and Royal had intermediate values (0.51 ± 0.14 and 0.55 ± 0.06 respectively) while for *Spanish white* and *Dwelly*, N_2 fixation started to decrease earlier while soil was drying with FTSW threshold values of 0.61 ± 0.25 and 0.72 ± 0.07 respectively. The steady part of NARA is not always close to 1 because ARA measurement were not steady. Nitrogen fixation took time to set up in some repetition and NARA was very low during the first few days of experiment. Normalization procedure have smooth this variation but this have led to some variability in the NARA value for FTSW = 1.

Table 8 : threshold (BP) of Fraction of transpirable soil water (FTSW) for the decrease in normalized acetylene reduction activity (NARA) and normalized transpiration rate (NTR) in response to soil drying of 7 *C. arietinum* accessions. β_1 is the slope of the NTR or NARA decrease after the threshold. Thresholds and slopes are followed by different letters based on the 95% confidence interval.

Accession	NTR vs FTSW			NARA vs FTSW		
	BP \pm CI	$\beta_1 \pm$ CI	R^2	BP \pm CI	$\beta_1 \pm$ CI	R^2
Billy	0,56 \pm 0,08 ^a	1,63 \pm 0,24 ^a	0,85	0,35 \pm 0,23 ^{abcd}	1,79 \pm 1,48 ^{abc}	0,28
Orion	0,56 \pm 0,07 ^a	1,84 \pm 0,24 ^a	0,91	0,51 \pm 0,14 ^{bcd}	1,90 \pm 0,57 ^{abc}	0,69
Dwelly	0,54 \pm 0,05 ^a	2,00 \pm 0,19 ^a	0,96	0,72 \pm 0,07 ^e	1,62 \pm 0,13 ^{bc}	0,96
Myles	0,50 \pm 0,04 ^a	1,91 \pm 0,19 ^a	0,95	0,31 \pm 0,09 ^{abcd}	3,27 \pm 1,25 ^{ac}	0,65
Royal	0,56 \pm 0,06 ^a	1,81 \pm 0,21 ^a	0,93	0,55 \pm 0,06 ^{bd}	1,86 \pm 0,25 ^{abc}	0,92
Spanish white	0,52 \pm 0,06 ^a	2,07 \pm 0,25 ^a	0,91	0,61 \pm 0,25 ^{bcd}	1,46 \pm 0,43 ^{bc}	0,48
CA04	0,52 \pm 0,05 ^a	1,87 \pm 0,22 ^a	0,93	0,12 \pm 0,15 ^a	5,43 \pm 9,37 ^{abc}	0,14

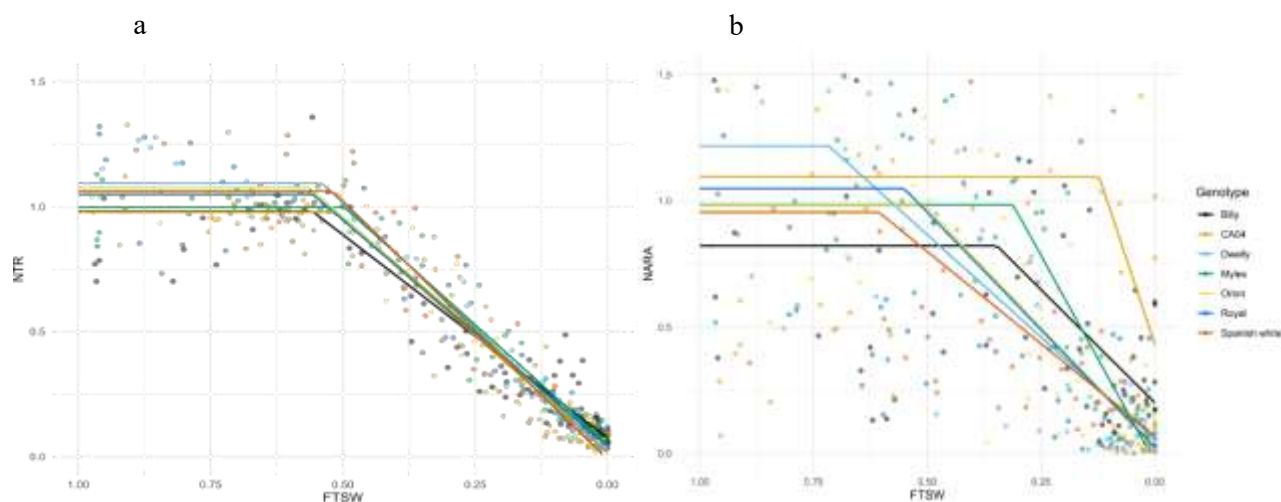


Figure 17: Plots for all seven *C. arietinum* accessions of (a) normalized transpiration rate (NTR) vs. fraction of transpirable soil water (FTSW) and (b) normalized acetylene reduction activity (NARA) vs. fraction of transpirable soil water (FTSW). Solid Lines represent the result of segmented regression for each accessions (see Table 1) and the existence of a significant breakpoint was tested through a Davis test

2. Variation of leaf traits

SLA values (Table 9) measured at the end of the experiment varied significantly among accessions ($P < 0.001$). Values for well-watered plants ranged from $153.04 \pm 25 \text{ cm}^2 \text{ g}^{-1}$ (*Spanish white*) to $325.11 \pm 14.7 \text{ cm}^2 \text{ g}^{-1}$ (*CA04*), and from $141.72 \pm 53.5 \text{ cm}^2 \text{ g}^{-1}$ (*Spanish white*) to 207.36 ± 23.7 (*Dwelly*) for plants under water deficit. Well-watered plants had higher values of SLA than plants grown under water deficit. This resulted in a significant difference of SLA between stressed and unstressed plants ($P < 0.001$). SLA under water deficit range between $7.40 \pm 34.9 \%$ to $37.26 \pm 5.9 \%$ lower than SLA under well-watered conditions but this difference was not significantly different among accessions.

LNC values (Table 9) ranged from $15.3 \pm 2.8 \text{ mg N g}^{-1}$ (*Spanish white*) to $26.5 \pm 11.9 \text{ mg N g}^{-1}$ (*Dwelly*) under well-watered conditions and from $12.0 \pm 3.1 \text{ mg N g}^{-1}$ (*Spanish white*) to $45.0 \pm 5.3 \text{ mg N g}^{-1}$ (*Billy*) under water deficit. LNC was significantly increased under water deficit ($P < 0.001$). LNC values under water deficit were up to $110.0 \pm 45.9 \%$ (*Royal*) higher than LNC values under well-watered conditions.

Table 9: Average Specific leaf area (SLA; cm².g⁻¹), Leaf nitrogen content (LNC; mg N g⁻¹) ± standard deviation (SD) of *C. arietinum* accessions. Traits were measured at the end of the dry down experiment on five water-stressed (WS) plants and three well-watered plants (WW). SLA and LNC change are the difference between WW and WS plant express as a percentage of the WW value. SD stands for standard deviation. Values followed by different letters are significantly different based of the analysis of variance.

Accession	Treatment	SLA (cm ² g ⁻¹)	SLA change (%)	LNC (mg N g ⁻¹)	LNC change (%)
Billy	WD	197,61 ± 18,2 ^{abc}	-14,01 ± 7,9 ^a	45.0 ± 5.3 ^{ef}	93,5 ± 25,9 ^{ab}
	WW	229,81 ± 23,8 ^{ad}		20.6 ± 5.0 ^{abde}	
CA04	WD	203,99 ± 19,3 ^{abc}	-37,26 ± 5,9 ^a	25.5 ± 3.4 ^{abdf}	19,5 ± 16,0 ^{bc}
	WW	325,11 ± 14,7 ^d		21.3 ± 6.2 ^{abde}	
Dwelly	WD	207,36 ± 23,7 ^{abc}	-11,91 ± 10,1 ^a	30.6 ± 8.9 ^{bdf}	15,5 ± 33,8 ^{ac}
	WW	235,39 ± 12,1 ^{ad}		26.5 ± 11.9 ^{abdf}	
Myles	WD	206,47 ± 15,9 ^{abc}	-25,05 ± 5,8 ^a	40.2 ± 11.9 ^f	94,4 ± 57,5 ^b
	WW	275,49 ± 8,64 ^{cd}		20.6 ± 6.3 ^{abde}	
Orion	WD	197,86 ± 24,3 ^{abc}	-23,68 ± 9,4 ^a	35.0 ± 7.1 ^{cdf}	72,1 ± 35,3 ^{ab}
	WW	259,26 ± 3,1 ^{bd}		20.3 ± 4.1 ^{ad}	
Royal	WD	205,23 ± 14,0 ^{abc}	-15,49 ± 5,8 ^a	37.8 ± 8.2 ^{df}	110,0 ± 45,9 ^{ab}
	WW	242,84 ± 31,7 ^{ad}		18.0 ± 4.0 ^{abc}	
Spanish white	WD	141,72 ± 53,5 ^a	-7,40 ± 34,9 ^a	12.0 ± 3.1 ^a	-21,7 ± 20,6 ^c
	WW	153,04 ± 25 ^{ab}		15.3 ± 2.8 ^{ab}	

3. Relationships between leaf traits and parameters of transpiration and N fixation dynamics

NARA threshold was negatively correlated to SLA measured under well-watered conditions ($r = -0.72$; $P < 0.1$) as well as positively correlated with the relative decrease in SLA observed under water deficit ($r = 0.82$; $P < 0.05$). Both correlations did not remain significant when results collected on *Vigna sp.* were included ($r = -0.22$; $P = 0.4$; $r = -0.34$; $P = 0.2$). However, the relationship between well-watered SLA and NARA threshold was still negative (Figure 18). Conversely, the insignificant relation observed between NARA threshold and LNC in *Vigna* was not observed in *C. arietinum*. Similarly, NTR threshold did not correlate to leaf traits neither to their variation. LNC was significantly positively correlated with SLA but only under water deficit conditions ($r = 0.47$; $P < 0.05$).

The NARA threshold did not correlate with the NTR threshold. Two accessions expressed a decrease in NARA prior to the observed decrease in transpiration (*Dwelly* and *Spanish white*). In the other accessions transpiration decreased at a higher FTSW than N₂ fixation.

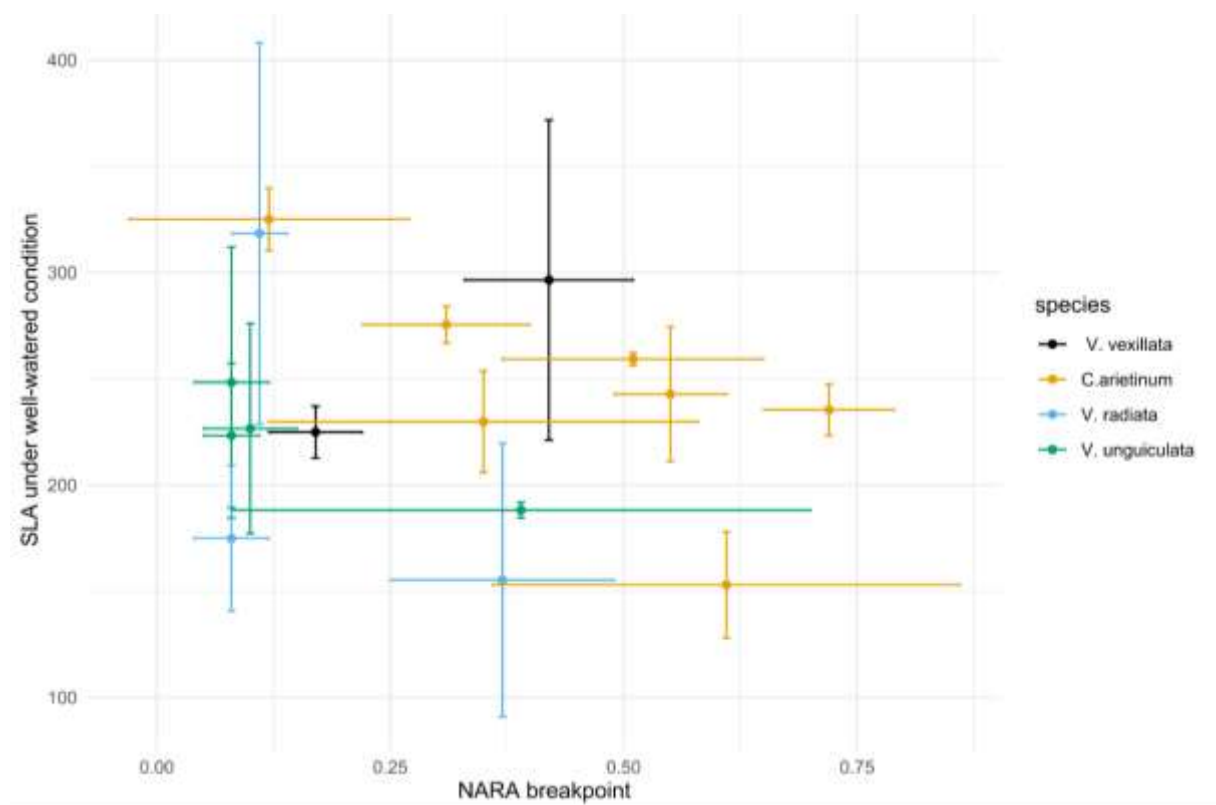


Figure 18 : Correlation between the FTSW threshold for the decrease in normalized acetylene reduction activity (NARA) and the SLA values of well-watered plants. Horizontal and vertical bars represents the 95% confidence intervals for the threshold values. Colors represent the 4 species studied (the three *Vigna* sp. of previous study and *C. arietinum*).

IV. Discussion

1. *A very early response of transpiration to soil drying for C. arietinum*

Previous observation on chickpea reported a FTSW threshold for transpiration decrease between 0.34 and 0.47 (Zaman-Allah, Jenkinson and Vadez, 2011; Sivasakthi *et al.*, 2017) which is quite low compared to what was observed in our experiment (0.54). Our previous observations on *Vigna* sp. ranged between 0.23 to 0.33. Apart from soybean which expresses thresholds between 0.2 and 0.3 (Hufstetler *et al.*, 2007; Sadok *et al.*, 2012); all other legume species (chickpea, lentil, peanut and common bean) had been reported to have thresholds above 0.3 (Bhatnagar-Mathur *et al.*, 2007; Devi *et al.*, 2009, 2013; Zaman-Allah, Jenkinson and Vadez, 2011; Ghanem *et al.*, 2017). With a threshold average above 0.5, those chickpea accessions place the chickpea among the most sensitive species tested with peanut (average at 0.54 and 0.66 ; Bhatnagar-Mathur *et al.*, 2007; Devi *et al.*, 2009).

The main expected benefit of early transpiration limitation with soil drying is to restrict water losses during the crop cycle so that severe late-season drought may be avoided. Increasing availability of water for the crop at the end of the season enhances the chances of sustained crop physiological activity, and hence, to decrease the impact of water deficit on reproductive growth. However, the relative advantage or disadvantage of early stomatal closure in the field might depend on the duration of drought and its timing. Reduced stomatal conductance is also a main limitation to photosynthesis under drought conditions in cowpeas (Medrano, 2002; Singh and Raja Reddy, 2011; Carvalho et al., 2019). Thus all accessions tested could be characterized as “water-saver” with an early response of transpiration to soil drying.

2. *A high sensitivity of N₂ fixation to soil drying in C. arietinum.*

Some of the experimented accessions exhibited N₂ fixation remarkably tolerant to soil water deficit (threshold at 0.12) while other were much more sensitive (0.92). Yet, the only accession expressing a FTSW threshold under 0.3 was CA04 for which data were poorly represented by the segmented regression ($R^2 = 0.14$). In previous studies, common bean genotypes had a NARA threshold ranging from FTSW = 0.21 to 0.4 (Devi *et al.*, 2013); in peanut the threshold ranged from FTSW = 0.28 to 0.59 (Devi, Sinclair and Vadez, 2010). With four accessions having a FTSW threshold over 0.5, chickpea show a relatively early decrease for nitrogen fixation under soil drying and would be more sensitive than *Vigna sp* from our previous study.

As in previous results, N₂ fixation was not identified as the mechanism most sensitive to water deficit, as it decreases after gas exchange for most accessions studied here. In addition, NARA and NTR threshold were not correlated. In the previous study on *Vigna sp.*, high NARA threshold were associated with small increase in LNC. Therefore, it seemed that accumulation of N in leaves is a consequence of the maintenance N₂ fixation over a longer period. Such correlation was not confirmed with chickpea accessions perhaps because of the lack of data or the poor quality of NARA measurement.

3. *Leaf traits as indicators of C and N metabolism changes under drought*

We observed a decrease in SLA under drought for all accessions. A decrease in SLA under drought have also been found in groundnut (Aparna *et al.*, 2018). However, this contrast with *Vigna sp.* results. Usually, smaller SLA are regarded as adaptations to drought stress (Abrams, 1994; Pita and Pardos, 2001; LaForgia *et al.*, 2018), and SLA generally decrease under drought (Liu and Stützel, 2004; Jongrunklang *et al.*, 2008; Painawadee *et al.*, 2009). SLA adjustment to drought is generally related to leaf thickness. But such adjustment may impair photosynthesis capacity and overall productivity. Change in SLA in response to drought was identified as the a key predictor of above-ground biomass production within grassland population (Vitra *et al.*, 2019).

In this study, a higher N concentration in leaves was observed for most accessions when plants were submitted to soil water deficit except for Spanish White which expressed the lowest LNC value. This was consistent with *Vigna sp.* data. Therefore, SLA and LNC values were well correlated only under water deficit and their change under drought were not correlated. This accumulation of N in leaves under drought was already observed in cowpea in tandem with decrease in C stock which could be explained by a metabolism regulation to balance the production of N and C containing metabolites (Goufo *et al.*, 2017) . Increased LNC under water deficit would also be coherent with N₂ fixation being less sensitive to soil drying than transpiration for most accessions. However, this cannot be verified in all accessions.

Average value for SLA and LNC under well-watered conditions did not differ between *Vigna sp.* and *C. arietinum*. Therefore, the increase or decrease in SLA between well-watered and water-stress condition may be a key trait indicating various soil drying sensitivity at the inter-specific level.

V. Conclusion

Finally, our findings were consistent with a classification of *C. arietinum* accessions as “conservative species” based both on their leaf traits and dynamic responses of transpiration and N₂ fixation to soil drying. The decrease in SLA under drought is known as a way to save water that is consistent with the general behavior of chickpea identified in this study. Chickpea transpiration and nitrogen fixation responded faster to soil drying than *Vigna sp.* and most other species previously tested. This may allow to save water to restrict water losses during the crop cycle so that severe late-season drought may be avoided. This behavior can be related to leaf thickness adjustment.

List of Acronyms

LNC: leaf nitrogen content (mg N.g⁻¹)

FTSW: Fraction of transpirable soil water (L water.L⁻¹ soil)

NARA: Normalized Acetylene Reductase Activity (no unit)

NTR: Normalized transpiration rate (no unit)

SLA: Specific leaf Area (cm² g⁻¹)

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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CHAPTER 5. *GENERAL DISCUSSION*

A. Overview and main results of the thesis

I. Synergies or trade-offs between water stress resistance, crop production and desired support agro-ecosystem services

Managing pulse crops for multifunctionality requires knowledge of how services interact (Spake *et al.*, 2017; Turkelboom *et al.*, 2018) and how those interactions are influenced by species identity and diversity (Carpenter *et al.*, 2009). Management aiming at maximizing only one service often has negative impacts on other services and on biological and physical diversity (Bennett, Peterson and Gordon, 2009). The same factor can drive a change in multiple services, leading to co-occurring or “bundled” services which are governed by ecosystem components (Bennett, Peterson and Gordon, 2009; Finney, White and Kaye, 2016). Crops, being major components of agro-ecosystem, embody the main agro-ecosystem services trade-offs and synergies. Ecosystem services in agriculture are essentially those supporting or regulating production services, such as the three services we have targeted to support food production, by improving soil fertility, climatic limitation or pest regulation (Jordan *et al.*, 2011). Quantifying agro-ecosystem services as if they were independent from each other might turn out to be pointless. We chose to focus on agro-ecosystem properties which characterize biophysical processes and agro-ecosystem functioning. The analysis of the relationships among them allowed to highlight contrasting “strategies” among pulses and the main axes of agro-ecosystem properties variation within this peculiar group of species. In a second step, our field analysis allowed us to highlight agro-ecosystem properties bundles and antagonisms as revealed by the comparison of values measured in a comparable environment.

1. *Pulses species and varieties incidence on agro-ecosystem property suites*

Analyses from chapter 2 and 3 helped deciphering relationships among agro-ecosystem properties and highlighting bundles and trade-offs between them.

Within the large set of legume species studied in the second chapter of this thesis, PCA's first axis sorted species by their productive ability, thus identifying species which were selected under highly productive environments such as fertilized and irrigated cropping systems coupled with low competition due to recurrent disturbances (mechanical or chemical destructions). A second axis sorted species with varying degrees of sensitivity to competition according mainly to their ability to intercept light. Finally, the third axis may partially reflect species adaptation to stressful environments with low levels of nitrogen and water. These findings may have revealed that the trade-offs between pulses' agro-ecosystems properties were somewhat consistent with the Grime's CSR triangle theory, which predicts that plant strategies are selected according to environmental gradients of resource availability, stress and disturbance (Grime *et al.*, 1997; Grime, 2009). Agro-ecosystem

are ruderal environments: pulses as crop species have a peculiar evolutionary history compared to uncultivated species due to their breeding background. Even if they are less important than cereals in human nutrition, pulses might have been managed under non-limiting conditions (irrigation and fertilization) compared to wild species and would exhibit high grain production levels compared to species that were predominantly used for feed. Thus, it is more likely that agro-ecosystem properties trade-offs mostly highlighted classification of competitive ability inside ruderal strategies. Yet, species that were considered here came from a wide range of geographic origins and have therefore been subject to a great diversity of climates and farming practices along their evolutionary history, leading to contrasted adaptations to stressful conditions.

In the current context of climate change and reduction of resource availability, species that are able to maintain relatively high productivity under stressful conditions could be valuable for the provision of food. Nitrogen fixation by legumes is expected not only to support plant N demand in soils that are deprived of N, but also to contribute to improving soil fertility through the mineralization of crop residues such as senescent leaves, stems, or roots. Among the many services cover crop can provide (Blanco-Canqui *et al.*, 2015), legumes are sometimes used as cover crops to benefit from nitrogen fixation while lowering weed pressure (Reddy, 2001; Hiltbrunner *et al.*, 2007) The interaction between cover capacity and yield is rarely discussed.

Our field experiment allowed to analyze more precisely antagonisms and independencies and co-occurrence among agro-ecosystem properties within pulses species. The first properties “bundle” included yield properties which can be related to the primary production services. Another set included crop maximum biomass, LAI, crop cover, which have been associated to weed competitiveness in the second chapter. Other studies associated crop biomass to the prevention of nitrate leaching, and aboveground biomass N, but showed it negatively impacted inorganic N availability and corn yield in the subsequent cropping season (Finney, White and Kaye, 2016). Additionally, this bundle appears to be independent of yield properties. Delayed senescence, by prolonging photosynthesis, is usually expected to contribute to increasing the yield of high-carbon crops such as cereals (Gregersen *et al.*, 2013). However, other studies suggest that the stay-green trait may have limited value for high N species (Ismail, Hall and Ehlers, 2000; Kumudini, 2002) as its value lies in an improvement of remobilization capacity. This corroborates the absence of relation we found between yield and duration of ground cover. We also identified an important trade-off which is the incompatibility between high yield and high nitrogen fixation ability among the pulses species studied. Results also showed that Ndfa at flowering and Ndfa at harvest in grain tissue varied almost independently. Other multiple species comparison studies confirmed the negative relation between biological nitrogen fixation and seed yield (Jones and Olson-Rutz, 2018; Liu *et al.*, 2019; Mesfin *et al.*, 2020)

More importantly, those results highlighted, along with other studies (Guinet, Nicolardot and Voisin, 2020), the contrasting performance of each species or varieties. Consequently, when considering the inclusion of a pulse crop in their cropping systems, farmers should first and foremost have defined their objectives in terms of service delivery. Used as non-cash crop (e.g. as cover crop) plant resources in agro-ecosystems, legumes can improve supporting services such as weed mitigation or nitrogen nutrition (Vrignon-Brenas *et al.*, 2018). On the other hand for cash crop the primary production services might be more important than supporting services. Cultivar choice and hence breeding have often been proposed as major tools to improve crop performance under low-input and organic farming conditions (Bueren and Myers, 2011; Lammerts van Bueren *et al.*, 2011; Kiær, Skovgaard and Østergård, 2012). Hence, our study should facilitate the selection of pulses species according to the expected objectives. The understanding of trade-offs between agro-ecosystem properties should help to optimize crop diversification at field or farm scale to stabilize the rate and quality of agro-ecosystem services delivery. However, effective ecological intensification still requires an understanding of the relationships between agro-ecosystem management and the stability of the multiple services provided by these crops (Bommarco, Kleijn and Potts, 2013). This should allow using crop diversity to increase synergistic effects between stress resistance, crop production and desired agro-ecosystem services (Degani *et al.*, 2019)

2. Water deficit influence on agro-ecosystem properties rate and stability

In the fourth chapter of this thesis, the response of transpiration and nitrogen fixation dynamic to progressive soil water depletion were compared for different *Vigna sp.* and *Cicer arietinum* accessions.

The initiation of the decrease of the transpiration rate with soil drying is usually interpreted as the result of a stomatal regulation associated with decreasing soil water availability (Ray and Sinclair, 1997). The main expected benefit of early transpiration limitation with soil drying is to restrict water losses during the crop cycle so that severe late-season drought may be avoided. Increasing availability of water for the crop at the end of the season enhances the chances of sustained crop physiological activity, and hence, to decrease the impact of water deficit on reproductive growth. However, the relative advantage or disadvantage of early stomatal closure in the field might depend on the duration of drought and its timing. Reduced stomatal conductance is also a main limitation to photosynthesis under drought conditions (Medrano, 2002; Singh and Raja Reddy, 2011; Carvalho *et al.*, 2019). Based on the set of accessions tested in our experiment, there seem to be little variability of this trait across the *Vigna* genus and even less within *C. arietinum* species. Our study showed that *Vigna* species are very insensitive to soil drying compared to *C. arietinum* and most species tested in other studies. Thus all *Vigna* accessions tested could be characterized as “water-spenders” with a delayed response of transpiration to soil drying (threshold values at fraction of transpirable soil water

(FTSW) from 0.23 to 0.33), which would confer a drought tolerance to them when subjected to transient drought events. Conversely, *C. arietinum* could be defined as “water-savers” with an early response of transpiration (threshold values averaged at FTSW = 0.54) which would confer a drought tolerance to them when subjected to long term drought events. Further information on how those accessions perform in the field are still needed to draw a solid conclusion on the relation between drought tolerance and transpiration threshold; especially considering that drought tolerance could be conferred through many other traits since several factors and mechanisms (in shoots and roots) operate to enable plants to cope with water deficit.

However, in our experiments as in previous studies (Sinclair *et al.*, 2015), transpiration response to soil water deficit did not correlate with nitrogen fixation response. Previous studies on soybean have identified N₂ fixation as the mechanism most sensitive to water deficit, as it decreases before plant growth and gas exchange (Serraj, Sinclair and Purcell, 1999). This statement doesn't seem to be always true, as other studies have indicated that the opposite may be also true (Serraj and Sinclair, 1998; Devi and Sinclair, 2013). In our study, transpiration was the most sensitive mechanism for most accessions. In addition, some of the experimented *Vigna* accessions exhibited N₂ fixation remarkably tolerant to soil water deficit with five of the accessions out of nine having NARA threshold for FTSW below 0.11. This result confirms previous work on cowpea (Sinclair *et al.*, 2015), but contrasts with previous findings on other grain legumes. In previous studies, common bean genotypes had a NARA threshold above FTSW = 0.21 (Devi *et al.*, 2013); in peanut the threshold was not less than FTSW = 0.28 (Devi, Sinclair and Vadez, 2010). In a screen of a large set of soybean genotypes, Sinclair *et al.*, (2000) found only two genotypes with NARA thresholds as low as FTSW = 0.14. Only one accession of *C. arietinum* showed such tolerance with a threshold at 0.12, values of other accession ranged from 0.31 to 0.72.

Biodiversity, by allowing the co-existence of several species which could fulfill similar functions, but express different responses to perturbations, is recognized to enhance agro-ecosystem resilience (Walker, 1995). This concept is linked to the “insurance hypothesis” (Yachi and Loreau, 1999), which proposes that biodiversity provides an insurance, or a buffer, for the maintenance of crucial agro-ecosystem services against environmental fluctuations because different species respond differently to change (Degani *et al.*, 2019). Our result showed that nitrogen fixation and transpiration dynamics expressed various responses to soil drying. Our results does not allow to link those process to actual agro-ecosystem properties such as potential yield or maximal nitrogen fixed by those accessions. It was therefore not possible, based on these results only, to investigate how the regulation of such processes could affect agro-ecosystem properties. Yet, by using alternatively or concomitantly different cultivars known for their contrasting level of resistance to soil drying, farmers should be able to mitigate the impact of environmental perturbation (Bueren and Myers, 2011; Lammerts van Bueren *et al.*, 2011; Kiær, Skovgaard and Østergård, 2012).

II. Patterns of trait covariations and plant production strategies

1. *Traits structuring pulses diversity*

Species traits offer a potentially powerful tool for predicting effects of environmental change on community structure and agro-ecosystem functioning. Increasing performance and improving the selection of species or varieties would require prior identification of key traits clearly associated with the provision of target agro-ecosystem services (Lazzaro, Costanzo and Barberi, 2018) as well as a broad characterization of functional trait variation and trade-offs across species and environment.

In the second chapter of this thesis, we conducted a first meta-analysis over 43 pulses species, based on an extensive literature review. We did not include all species existing in this specific group of crops but we were able to cover a large interspecies diversity concomitantly with a satisfying trait characterization details. Therefore, we were able to quantify the overall pulse' interspecific functional diversity and highlighted general trade-offs. Our results showed trait covariation patterns consistent with those predicted and observed by common Plant Ecological Strategies Schemes (PESS) previously described in wild species (Grime *et al.*, 1997; Westoby, 1998; Saugier, 2001; Westoby *et al.*, 2002; Lavergne, Garnier and Debussche, 2003; Quétier *et al.*, 2007). Indeed, we found that seed and leaf traits exhibited the greatest variability among the 43 pulse species.

Literature data revealed that leaflet size was a key parameter of legume functional diversity. The first PC axis was best explained by leaf size, leaf area and, especially, plant height, which is recognized as a good indicator of plant capacity for competitive dominance (Gaudet and Keddy, 1988; Westoby, 1998; Hodgson *et al.*, 1999) and thus a key trait for the design of successful crop mixtures. In general, cultivated plants have become larger since domestication and show erect and compact growth habits (Meyer, DuVal and Jensen, 2012). Further traits that might have changed during crop domestication can be relevant for plant performance in mixtures. For example, increased plant canopy height and leaf area after domestication (Milla *et al.*, 2014; Milla and Matesanz, 2017) suggest that crops are stronger competitors for light than their wild progenitors. Increased leaf size along domestication was identified as the main driver of increases in aboveground size (Milla and Matesanz, 2017). It is hypothesized that breeding and domestication may have led to a reduction in genetic diversity and might lead to increased phenotypic uniformity on group of crops (Bloomfield, Rose and King, 2014). Yet pulses species still express a great variability on those specific traits. Similarly, seed size, representative of a second axis of variation among pulses species, is generally larger in cultivated species (Smartt, 1978; Meyer, DuVal and Jensen, 2012). It is hypothesized that larger seed might produce individuals with larger organs (*i.e.* leaves) *via* cascading effects throughout ontogeny (Milla and Matesanz, 2017). Indeed, seed size is expected to be positively correlated with seedling biomass (Tamet *et al.*, 1996; Fayaud *et al.*, 2014), plant height and reproductive effort (Chapin, Autumn and Pugnaire, 2002). This would explain the partial association of seed size to the first axis of PCA.

Leaf functional traits have arguably received the most attention by ecologists and plant ecophysiologicalists. In particular, the ‘Leaf Economics Spectrum’ (LES) has been hypothesized and tested, as a suite of covarying leaf traits that can be used to describe plant functional diversity (Wright *et al.*, 2004). At one end of the LES are ‘resource conserving species’ that express low specific leaf area (SLA), low leaf nitrogen (N) concentration and low maximum photosynthetic rate (A_{\max}) (Wright *et al.*, 2004; Díaz *et al.*, 2016). At the opposite end of the LES are ‘resource acquisitive species’ that express high SLA, high leaf N and high A_{\max} . In turn, LES traits play an important role into applied research on multiple agro-ecosystem functions. In the search for a single trait that captures the core of this axis, specific leaf area (SLA) is a leading contender (Poorter and Remkes, 1990; Lambers and Poorter, 1992; Poorter *et al.*, 2009). However, our study shows that this trait contributed only marginally to the total trait variation among pulses through its contribution to the third PC axis. Part of pulse variability represented on the third PC axis was best represented by days to maturity and flowering. Yet, if evolution of plants/crops under agricultural selective pressures resulted in phenotypes that were larger and more effective competitors for light than their wild progenitors, studies show similar values in terms of mass-specific capabilities for leaf resource uptake and conservation (SLA and LDMC) (Milla *et al.*, 2014). Moreover, as previously observed by Tribouillois and collaborators (Tribouillois, Cruz, *et al.*, 2015) for herbaceous Fabaceae crops, the relationship between SLA and LNC was similar to that observed across a large diversity of wild species (Wright *et al.*, 2004; Díaz *et al.*, 2016) although LNC values of N-fixer species were on average higher than that of non N-fixer species.

The total percentage of variability explained by the first three PCs (61%) was relatively low compared to what is usually observed in wild species (Wright *et al.*, 2004; Díaz *et al.*, 2016). This could be attributed to the degree of missing data and/or the peculiarities of these cultivated Fabaceae species. Genetic bottlenecks (due to selection processes) and crop breeding for maximum monoculture yield have substantially reduced the genetic diversity of crops (Zeder, 2006). This reduction in genetic diversity might be leading to increased phenotypic uniformity, that is, identical phenotypes in different environments (Bloomfield, Rose and King, 2014). Genetic variability triggers resource use complementarity in mixtures (Zeller *et al.*, 2012; Zuppinger-Dingley *et al.*, 2014; Prieto *et al.*, 2015). Indeed, a recent study employing genetically divergent *Arabidopsis* accessions suggests that biodiversity effects in species mixture can be partly explained by a single chromosomal region (Wuest and Niklaus, 2018). Thus, while domestication and further breeding have reduced genetic diversity and increased homogeneity of phenotypes, achieving higher productivity in crop mixtures through increased niche complementarity might be compromised. Yet pulses species still show a great variability similar to wild species. This may be result from the reduced attention those species have received from scientists (Cuevas, 1992; Foyer *et al.*, 2016), and their exclusion from breeding efforts. The cultivation of neglected and underutilized species is increasingly recognized as a viable development strategy with benefits such as managing climate risk and enhancing agrobiodiversity

(Padulosi, Eyzaguirre and Hodgkin, 1999; Padulosi, Thompson and Rudebjer, 2013). It has been argued that evolution of plant phenotypes after domestication might partly account for such discrepancies between the behavior of wild and crop plants in mixtures (Chacón-Labela *et al.*, 2019). Indeed, while biodiversity effects have been solidly demonstrated in natural ecosystems (Tilman *et al.*, 2001; Hooper *et al.*, 2002; Cardinale *et al.*, 2006), the literature reports contrasting responses in mixtures of arable crops, ranging from positive (Reiss and Drinkwater, 2018) to negative (Snapp, Gentry and Harwood, 2010). Selection in diverse mixtures may therefore increase species coexistence and ecosystem functioning in natural communities and may also allow increased mixture yields in agriculture or forestry (Zupping-Dingley *et al.*, 2014). It has been shown that wild progenitors might also have useful traits for coexistence in mixtures (Chacón-Labela *et al.*, 2019). The compilation of available information regarding crop use, domestication history, and wild relatives can help to guide both *in situ* and *ex situ* conservation efforts to maintain a useful diversity. Comprehensive knowledge of the state of agricultural biodiversity is critical in guiding our efforts to promote, conserve and utilize our rich heritage of global food crops.

2. Trait co-variations and trade-offs

Our field experiment (Chapter 3 of this thesis) was performed on a smaller set of species but allowed to collect more complete and comparable traits observations. Unlike literature data, traits were measured under the same environment and management conditions, this allowed to highlight traits co-occurrence as well as individual trade-offs among traits.

In this second analysis, leaf traits remained an important variability driver, whereas seed size, associated to the first axis in literature, poorly contributed to the total variance across varieties and was decoupled from leaf size. In addition, leaf size was strongly associated to traits related to nitrogen acquisition (LNC, SNC). Alike LNC, leaves divided into small leaflets appear to be a frequent component of ecological strategies emphasizing a productive photosynthetic apparatus (Leavitt, Dobrenz and Stone, 1979). LNC covariation with SLA reminds the already described leaf economic spectrum (LES; Wright *et al.*, 2004) and could thus reflect a gradient in leaf photosynthetic capacity. Yet a high LNC could have other biological implications such as remobilization to the reproductive organs (Davies *et al.*, 2000; Schiltz *et al.*, 2005). Indeed, SNC was correlated with LNC in our experiment. Pulses are also known for their often nitrogen-rich seeds compared to other crop species (Sinclair and Wit, 1975; Hardwick, 1988). Thus, small leaflets occurring concomitantly with a high LNC could be important for reproductive strategy through remobilization.

Leaves also play a specially crucial role in carbon assimilation, water relations and energy balance (Ackerly *et al.*, 2002). Consequently, the biomass allocated to leaves can have a significant effect on plant resource acquisition. Here, investment in leaves (area based) seemed tightly linked to nitrogen accumulation in plants and reallocation to seeds. Our data showed a trade-off between LAR and LA.

This balance obviously depends on the total biomass produced per species. Yet, biomass invested in leaves (LMR), which contributed to the total variability of traits via the second axis of the experimental PCA, was not correlated with area-based leaf investment (LA, LAR). In theory, an increased LAR provides more leaf area for light capture per unit biomass (Brenes-Arguedas, Roddy and Kursar, 2013; Yin *et al.*, 2019). On the other hand, the present experiment highlighted that LMR together with SMR were correlated with traits related to growth rate (cover rate, degree day to maturity). Higher biomass allocation to leaves was related to a slow growth. Which is consistent with the third dimension of the LSH scheme related to the trade-off between “fast” traits that promote rapid resource acquisition and “slow” traits (Wright *et al.*, 2004; Díaz *et al.*, 2016; Volaire, 2018) and generally represented by SLA (Poorter and Remkes, 1990; Lambers and Poorter, 1992; Poorter *et al.*, 2009). In the present study, this trade-off seemed to be more related to the percentage of biomass allocated to leaf independently from the leaf area achieved.

3. *Traits variations between cultivars and growing conditions*

In our field experiments (chapter 3), each species was represented by three cultivars, allowing us to have a slight information on intra-species variance. The results showed that the total trait variance was mainly explained by the variance between species. Variation in intraspecific characteristics is generally considered of marginal importance compared to differences in interspecific characteristics (McGill *et al.*, 2006; Hulshof and Swenson, 2010). However, cultivated plants/crops, and among them pulses (Ruelle *et al.*, 2019), often exhibit a high intraspecific variation in functional traits, reflecting both heritable genetic variation and phenotypic plasticity. The small number of varieties considered here may have not been able to highlight such diversity. Several recent studies have shown that intraspecific variation in traits accounts for a substantial proportion of overall plant community variability (Jung *et al.*, 2010; Albert *et al.*, 2011; Roscher *et al.*, 2015; Siefert *et al.*, 2015). The importance of intraspecific trait variation is expected to play a key role in the management of agro-ecological processes. The trait-based approach may be easier to implement in crop species where varieties origin and genetics are often better described and accessible, compared with the huge genetic variation encountered in populations of spontaneous species. When comparing mixtures with equal cultivar richness, functional trait diversity measured as the additive trait range is generally positively associated with biomass production and over-yielding. For example, cultivar mixtures of perennial ryegrass can lead to improved forage production when specific functional traits are optimized within mixtures (Lowry *et al.*, 2020). Yet the response of one individual cultivar can vary depending on the other cultivar in the mixture and that this plastic response can significantly influence productivity in mixtures (Dahlin *et al.*, 2020). This, concomitantly with trait plasticity across environments, can explain the difficulty of finding general “mixture effects”.

Through functional traits, researchers usually seek to make generalizable predictions across organizational and spatial scales (Adler *et al.*, 2013). Valuable knowledge has been obtained on the

functional diversity of managed species from studies that use data collected in various environments and with different managements (Gunton, Petit and Gaba, 2011; Yu *et al.*, 2015; Kunstler *et al.*, 2016; Isaac and Martin, 2019; Guiguitant *et al.*, 2020). However, field measurements sharing similar environment as in our third chapter deliver a local picture of functional diversity which variations can be solely attributed to genotypic determinants. Both of our approaches (meta-analysis and field measurement) showed similar traits covariation patterns. Species rankings on the PC axis summarizing the variation of architectural traits were fairly consistent with literature ranking. This was consistent with several studies that have demonstrated that despite the significant plasticity of most plant traits across environments, the classification of species for a given trait remains fairly consistent in space and time (Garnier *et al.*, 2001; Kazakou *et al.*, 2014; Tribouillois, Fort, *et al.*, 2015). However, rankings on the axis summarizing SLA and LNC variation diverged between the two data sets in our study. This contrast mainly concerned warm-season species, particularly *Vigna* sp. for which divergence between the two data sets were also noted in terms of phenology. Number of days required to reach maturity was generally lower in literature than in experimental data. This comparison between local measurements and literature averages suggests that some traits are more plastic than others and therefore contribute differently to frame legume diversity depending on the conditions of observation. Other studies have reported a significant effect of trait plasticity on species ranking across environments, particularly with respect to traits associated with light acquisition and nitrogen nutrition (Lipowsky *et al.*, 2015; Siebenkäs, Schumacher and Roscher, 2015; Keenan and Niinemets, 2017). These inconsistencies may impair the use of simple aggregation functions (means, medians, etc.) for generalized trait-based approaches at the species level.

In chapter 4, we investigated intra-species variability of leaf trait as well as trait plasticity to a progressive soil drying. SLA and LNC were chosen because of their relation to the leaf economic spectrum which characterize species resource acquisition strategies. High SLA and LNC are known, in ecology, to lead to an opportunistic, water-spender strategy (Wright *et al.*, 2004). Both *C. arietinum* and *Vigna* sp. expressed a high and significant intra-species variability for both traits while species means were similar. Drought impact on SLA differed among the accessions tested. We observed an increase in SLA under drought for most *Vigna* accessions and a decrease for all *C. arietinum* accession. Usually, smaller SLA are regarded as adaptations to drought stress (Abrams, 1994; Pita and Pardos, 2001), and SLA was found to decrease under drought (Liu and Stützel, 2004; Jongrunklang *et al.*, 2008; Painawadee *et al.*, 2009). Increase in SLA under drought could result from changes in carbon allocation in the plant. Studies have indicated that drought could induce a reduction of carbon allocation to leaves possibly related to their strategy to allocate resources to belowground parts (Wellstein *et al.*, 2017). Increasing SLA would allow the plant to compensate lower carbon allocation to leaves, while maintaining a given leaf area (Wang, Hawkins and Letchford, 1998; Palmroth *et al.*, 1999; Wellstein *et al.*, 2017). Similarly, LNC increase for almost all *Vigna* sp. and *C. arietinum* accessions when plants were submitted to soil water deficit. This

accumulation of N in leaves under drought was already observed in cowpea and could be explained by a metabolism regulation to balance the production of N and C containing metabolites (Goufo *et al.*, 2017). Conversely, some results suggest that drought-related decreases in leaf nitrogen concentration limit photosynthesis during recovery (Heckathorn, DeLucia and Zielinski, 1997). Moreover, high LNC is usually associated to the “stay green” type. More especially in case of terminal drought, it has been shown that if LNC declines to a critical threshold, leaf senescence will set up (Borrell and Hammer, 2000; Borrell, Hammer and Van Oosterom, 2001). Thus the contrasted LNC responses to drought might be related to the fact that *C. arietinum* accessions started their leaf senescence early during soil drying while *Vigna sp.* accessions did not shed leaves until the very end of the experiment.

III. From traits to agro-ecosystem services

Species richness has been shown to explain only 3% of the variation in multifunctionality (Finney *et al.*, 2017). This outcome suggests that species number is less important to multifunctionality than the variety of traits present in a crop mixture. Agronomic research have usually focused on using traits to understand how biodiversity in agricultural systems responds to environmental conditions and land management, rather than on understanding how biodiversity impacts agro-ecosystem services (ie. Vos, Putten and Birch, 2005; Wellstein *et al.*, 2017). Functional approaches are based on the assumption that agro-ecosystem production services and supporting processes can be best explained by a set of species traits expressing their response to management and environmental condition than by species richness.

1. *Important traits for prediction of agro-ecosystem properties*

A tricky question rising from functional trait approach is the selection of relevant traits to predict plant functioning in an environment and which could be generalized across taxa. The standard plant ecological strategy schemes (PESS) previously described for wild species have provided evidence of several traits that can be generalized to many species, easily measured, and more or less linked to biological functioning in terms of resource use and acquisition (Grime *et al.*, 1997; Westoby, 1998; Saugier, 2001; Westoby *et al.*, 2002; Lavergne, Garnier and Debussche, 2003; Quétier *et al.*, 2007). Since then, those traits among others, have been used to explain agro-ecosystem properties. Relevant traits may vary between species. Experiments are needed for individual taxa to determine the key traits for specific processes prediction. Our analysis allowed to identify the most important traits (among those measured) for prediction of individual agro-ecosystem properties as well as multifunctionality.

As noted earlier, some agro-ecosystem properties co-occurred in pulse species which allowed us to construct clusters of varieties based on agro-ecosystem properties. Some traits values were found

strongly related to this clustering. Leaf characteristics and especially LNC were crucial in describing pulse diversity. We also highlighted that LNC was an important predictor of pulses agro-ecosystem properties in our specific context. Other traits such as LAR, total nitrogen, seed size and cycle length were also important. This partially confirmed the results obtained in chapter 2 where similar traits were found helpful to predict agro-ecosystem properties mean values for a wider range of species across multiple environments. This second approach allowed to highlight generic linkage because we compared mean values of traits and properties measured in different condition and on different individuals. On the contrary, field analysis allowed to draw a more precise map of the traits-properties relations. It is difficult to define one ideal combination of plant functional traits which would maximize all agro-ecosystem properties. TSW participated in the prediction of most agro-ecosystem properties in chapter 2 and was still relevant to describe functional differences between species associated to each agro-ecosystem properties cluster in chapter 3. Differences in growth cycle length also appeared to be discriminant for properties values in each approach. The meta-analysis in chapter 1 identified leaflet length and SLA as important discriminants between species functional groups while they were found insignificant in chapter 3. Yet, chapter 3 analysis highlighted the importance of the trade-offs between LAR and LMR. Those differences may only be a result of different data precision as LMR and LAR were not available in our literature survey. LNC was also strongly related to agro-ecosystem properties in both study so as plant nitrogen content.

2. Traits to agro-ecosystem properties

Trait were either related to single agro-ecosystem properties in chapter 2 or to groups of co-varying properties in chapter 3. In that way, we were able to identify commonly described linkages. However, we found that prediction of agro-ecosystem properties did not always rely on the traits generally used for the quantification of each axis alone. Leaflet length was found more relevant than plant height to predict competitive ability against weeds and days to maturity a better predictor than SLA for prediction of pulse productivity as indicated by CART results for prediction of biomass and grain yield performances in chapter 2.

LNC together with seed weight were good predictors of grain yield in the second chapter. Yet, results of second chapter showed that a high LNC value does not translate into higher values for 'yields properties' but is preferentially associated with a high nitrogen fixation (which was antagonist to yield). In chapter 2, LNC was also associated to WUE which is closely related to yield. LNC at anthesis and grain yield under drought have been previously found to be strongly linked (Borrell and Hammer, 2000). Indeed, when nutrient uptake is limited by water availability (Chapin, 1993), remobilization of N from vegetative tissues becomes particularly important for grain growth (Ta and Weiland, 2010).

Moreover, high LNC is usually associated to the “stay green” types, which have proved their value in case of terminal drought (Borrell and Hammer, 2000; Borrell, Hammer and Van Oosterom, 2001). More generally, N plant status is therefore closely related to the longevity of photosynthetic organs (Kikuzawa, 1991; Reich, Walters and Ellsworth, 1997). This is consistent with our findings in chapter 3, which indicate that long cycles and high nitrogen content at maturity are both related to delayed senescence and maintenance of N₂ fixation along pod filling. In addition, high nitrogen fixation was also achieved by late maturing species in the second chapter. This result is in accordance with previous studies (Kumar Rao and Dart, 1987; Piha and Munns, 1987). These factors account for the superior symbiotic performance of late maturing bean cultivars. Our results also highlighted small leaflets as a secondary trait involved in high nitrogen fixation which is consistent with the negative correlation found between LNC and leaflet length in our third chapter and confirmed LNC as an important trait to predict nitrogen fixation ability.

Our first results showed that high biomass species had high SLA. High SLA might allow (given favorable growth conditions) a shorter payback time on a gram of dry matter invested in leaf (Poorter, 1994), therefore improving production rate. Investment in leaf area concomitantly with a low biomass investment in leaves was revealed as a relevant predictor of high value for the second properties ‘bundle’ related to LAI biomass and soil cover at flowering but were also important for predicting N₂ fixation at flowering. Indeed, biomass is hardly dissociable from nitrogen fixation as it is a process driven by N demand (Hartwig, 1998; Hartwig *et al.*, 2002). This leaf investment ‘strategy’ was also associated with rapid senescence and low yield. Inversely, biomass investment in leaves and a short cycle were associated with low yield, nitrogen fixation and LAI.

In our study, yield reduction due to weed was mostly found correlated with LAI indicating that light competition might be an important factor. Many ecological studies have pointed out that competition is mostly encountered in highly productive environments. Indeed, when soil resources are more abundant, light might become the limiting resource (Gaudet and Keddy, 1988). LAI was found higher for large seeded species with hypogeal germination in the second chapter. As for biomass, grain yield and nitrogen fixation, LAI can be related to seedling vigor. In addition, our results show that yield was less likely to be impacted by weeds for early maturing species with small leaves. Small leaves are less expensive to produce (Poorter, 1994) and might be more efficient for light interception as their production reduces the risk of self-shading (Smartt, 1978). Beside leaflet size, early growth can provide an important advantage to the crop for light acquisition as well as for nitrogen accumulation through a faster and deeper root growth (Bedoussac and Justes, 2010a).

3. *Trait variability as an indicator of the sensitivity of physiological processes to environmental conditions*

Trait response to environmental variation have been shown to vary greatly among and within pulses species. Our experiment on *Vigna sp.* and *C. arietinum* allowed to study if variation in leaf morphological traits in relation with soil drying were related to N₂ fixation or transpiration decline. High SLA and high LNC are known, in ecology, to lead to an opportunistic, water-spender strategy (Wright et al., 2004), thus we tried to relate their variation with accession response to soil drying.

Several hypotheses coexist on the mechanisms that could trigger N₂ fixation decline under water deficit: oxygen limitation, reduction in carbon pool, or regulation by N metabolism. It has been suggested that plant N status acts as a feedback signal which regulates N₂ fixation (Thavarajah and Ball, 2006; Gil-Quintana *et al.*, 2013). Yet, for *Vigna sp.* high NARA thresholds are associated with small increase in LNC which doesn't support the hypothesis that nodule activity is back-regulated by the accumulation of N in leaves. Therefore, it seems that accumulation of N in leaves is a consequence of the maintenance of N₂ fixation over a longer period. However, this correlation was not maintained when *C. arietinum* varieties were included even though it kept the same trend. LNC is therefore not significantly relevant to predict the N₂ fixation decline.

For *Vigna sp.*, SLA values under water deficit were correlated to the FTSW threshold of transpiration. This corroborates that early stomatal closure could have impacted carbon gain and modified the allocation to leaves sooner in accessions expressing a relatively high FTSW threshold to trigger stomatal closure. Therefore, the increase in SLA observed for most *Vigna* species might be explained by a reduced carbon allocation to leaves. For *C. arietinum*, SLA variation was associated to NARA threshold. The earlier N₂ fixation decreased the more SLA values dropped under drought.

In conclusion, variation of leaf trait under environmental change could hardly be related to the differences in accession physiological functioning. If some weak relations were found, they were not consistent across species. However, this did not invalidate the previous relation highlighted between LNC and nitrogen fixation or WUE.

B. Limits

The approach followed in this thesis has some intrinsic limitations. Ecologists have long understood that phenotypic differences between species play an important role in species coexistence. The promise made by “trait-based” plant ecology is a generalized prediction across community structure and environment which would be independent from taxonomy. This promise is a major reason for the increased popularity of this approach. Yet, some fundamental assumptions of trait-based ecology have not received sufficient empirical evaluation. Moreover, the application of such method to agro-ecosystem diversification may reveal some crucial knowledge gap.

I. Trait choice

The functionality of a trait is defined by its relation to plant fitness (Violle *et al.*, 2007). Almost all trait-based studies implicitly assume a monotonic relationship between trait values and fitness (Shipley *et al.*, 2016). Despite its foundational importance, one of the essential criticisms made to trait-based approach is the practical difficulty of documenting trait–fitness relationships, which make the approach rely either on proxies of fitness. Fitness in natural communities is defined mainly by growth, dispersion, survival and reproduction of individuals (Conway, 1987). Such definition of fitness makes it generally meaningless in agriculture. Agro-ecosystem values rely on other properties; productivity, stability but also sustainability, social value or equitability (Conway, 1987). Within agricultural selection, production of harvestable yield is the prime consideration for fitness (Jackson, 1997). Yet, even when seed is the harvestable unit, choosing yield rather than survivorship in the next generation for selection will have fundamentally different implications for plant growth and resource acquisition. Consequently, traits qualified as “functional” in ecological studies may not be of prime interest in agro-ecosystems. Additionally, given the large number of functional traits that are measured on plants, the dimensionality of plant form and function is potentially vast. Many traits are redundant (Laughlin, 2014). The intrinsic dimension of a multi-trait data set can be informally described as the minimum number of parameters or latent variables needed to describe it (Lee and Verleysen, 2007). Ecologists strive to determine the dimensionality of plant trait space for predicting species responses to environmental conditions (Grime *et al.*, 1997; Westoby *et al.*, 2002; Wright *et al.*, 2004). Eliminating the taxonomic focus on species identity frees us to evaluate the responses of traits to the environment and the effects of traits on ecosystem processes (Lavorel and Garnier, 2002). This shift dramatically reduces the dimensionality and complexity of the problem and vastly increases our ability to generalize across ecosystems and transcend taxonomic and geographic boundaries. There appears to be a tractable upper limit to the dimensionality of plant traits, including multiple correlated traits does not bring in any benefits and including more than eight traits leads to diminishing returns (Laughlin, 2014). The strength of the correlation between trait values and fitness or an agronomic equivalent of fitness, should guide our choice of traits.

In our third chapter, data availability led to some degree of incompleteness in the database where some traits or species were more documented than others. We tried to overcome this weakness by imputing data through probabilistic principal component analysis, although we acknowledge that this estimation might have induced bias for the prediction of agro-ecosystem properties. Ecological studies have focused on a limited number of traits, mainly because they were relatively easy to measure. Specific leaf area, seed size, and a few others are commonly measured (Kattge *et al.*, 2011; Shipley *et al.*, 2016). Agronomic studies, as those we reviewed for second chapter data collection, focused on different set of traits. Within the 329 references reviewed for second chapter data

collection including traits collected in the TRY database (Kattge *et al.*, 2011), seed size, LNC and SLA were clearly underrepresented compared to maturity or flowering times, TSW, oil or protein seed content and HI. This marks a clear divergence of objectives between studies which aimed at understanding mechanisms for growth, maintenance, survival and reproduction and those that focused on mechanisms promoting high or qualitative harvestable yields. The list of traits measured in chapter 3 was more complete, however the weak contribution of most of traits to the PCA axes might indicate those traits were not the best choice for pulses functional description. Moreover, time consuming or expensive measurements were obviously excluded from this work (both experimental and based on literature review) as they were measured only in very few studies for only one or two species. The focus on “cheap” traits is reasonable to satisfy the requirement for generality. However, an easily measured trait is not necessarily an important one and in many case the degree of importance of most traits will be context specific (Shipley *et al.*, 2016). Potentially every phenotypic trait of an individual will have functional consequences. Volaire *et al.* (2020) suggested trait-based approaches should not mistake traits suitable for pattern approach and traits suitable for process approach. Many standard "functional" traits from predictive model approaches such as SLA and LDMC are used to quantify the response to drought, rather than more reactive traits that are more closely related to drought. (Voltaire, Gleason and Delzon, 2020). Additionally, relationships among traits may differ among different groups of plants (Osnas *et al.*, 2018). So, it is unsatisfying to rely on general assumptions or out-of-context studies of cause-effect relationships. We need better guides to trait selection for different types of agronomic or ecological questions.

Specifically, when considering pulses species, their inherent capacity to fix N is a key agronomic expectative. In every chapter of this thesis, we tried to relate plant morphological attribute to this ability as well as with its regulation under drought. Even though we were able to measure more traits in chapter 3 than in the literature review (chapter 2), such as LNC or biomass partitioning, the variation in the amount of nitrogen fixed is not only plant dependent and could be attributed to nutritional factors, environmental conditions, rhizobia strains or other host characteristics such as root traits (Reichardt *et al.*, 1987; Nambiar, Rupela and Kumar Rao, 1988; Hardarson *et al.*, 1993). Since the data collected focused on aboveground plant traits, an incomplete picture of what drives nitrogen supply might have been obtained. Some studies also showed an allocation of 11–14 kg N fixed to belowground dry matter in indeterminate legumes, which represents half the amount of total aboveground plant dry matter (Carranca, Torres and Madeira, 2015). This finding demonstrates that investigation relying only on shoot N underestimates overall ability of pulses to fix atmospheric nitrogen and improve soil fertility. Alternatively, practical limits arise when, for some traits, there is a trade-off between their ecological value and their technical management. As an example, in a cultivar mixture, high plasticity in flowering time in response to growing conditions can buffer unpredictable environmental stresses and fasten evolution of locally adapted populations, but it can

result as well in heterogeneous ripening, which can be a problem for mechanical harvest (Costanzo and Bàrberi, 2014)

11. An unsatisfactory relation to agro-ecosystem services

Trait-based approach promise is to evaluate the responses of traits to the environment and the effects of traits on ecosystem processes (Lavorel and Garnier, 2002). Unprecedented efforts have been made by the scientific community and aiming at sharing trait information worldwide in order to improve the generality of our knowledge of trait interrelationships (for example, Wright *et al.*, 2004). Conversely, generalizable trait-ecosystem services relationships are still poorly elucidated. This is perhaps because not enough effort have been invested in looking for them and that establishing such relations requires more knowledge than we already have.

Despite the consensus around the general merit of accounting for ecosystem services, systematic guidance on what to measure and how is lacking. The existence of numerous conceptualizations and classification systems for ecosystem services has led to a plurality in the interpretation of ecosystem services and related terminology and definitions when it comes to applications (La Notte *et al.*, 2017). The absence of general definitions of ecosystem services make quantification very difficult. Current ecosystem services assessments often resort to biophysical proxies or even disregard services that seem difficult to measure (Olander *et al.*, 2018). This led us to rely on agro-ecosystem properties rather than estimation of services in this thesis. As agro-ecosystems are directly meant to serve humans, ecosystem services they deliver may be more easy to identify. Yet, few of the reported observations and experiments on the structure and functioning of agro-ecosystems have attempted to generalize regarding ecosystem structure and functioning. Moreover, there is a broader need for incorporating other social and socio-ecological traits into trait-based assessments of agro-ecosystems (Wood, 2018). Extrapolation of individual effects to the population level is informative, but focusing on service provision means that the spatiotemporal dynamics of service providers must be taken into account as well as possible interactions with populations of other species (Galic *et al.*, 2012). For many services, explicit considerations of the framework are essentials. Some models such as ‘Agro-ecosystem service capacity’ (ASC; Augstburger *et al.*, 2018), aim at understanding the relation between ecosystems, agro-ecosystems, and farming systems (or farms). This provides a framework for analyzing food production systems in their entirety, including their complex sets of inputs and outputs and the interconnections between their component parts (Augstburger *et al.*, 2018). The right balance in the amount of spatial and biological details is still to be found.

Furthermore, the multiple interactions, trade-offs and feedbacks between various service combinations have not received much attention (Bennett, Peterson and Gordon, 2009) and make single-factor optimization impossible (Sadras and Denison, 2016). The impossibility to manage services individually requires the help of models to efficiently implement provision prediction. It

might be necessary to break down general services into more manageable units and identify certain ecological “properties” that can reasonably be related to services in a given environment in order to relate them to traits that we suspect are important determinants of these abilities (Shipley *et al.*, 2016). There is a large literature relating traits to various ecological properties (Lambers *et al.*, 2006; Orwin *et al.*, 2010; Abalos *et al.*, 2019). In our study, even if we didn’t directly measure ecosystem services, the field measurement of underlying processes (namely agro-ecosystem properties) may have given insight on those interactions. Yet there is still a long way to ecosystem services. For instance, improvement of soil nitrogen fertility is not directly linked to the percentage of atmospheric nitrogen fixed and may depend on many other parameters. We selected WUE even though it is not the only driver of effective use of available water or drought resistance. Yet our objective was not to differentiate pulse species by their drought resistance but rather by their ability to maintain high production rate under drought, which is a more relevant trait for food production. We also largely ignored many agro-ecosystem services expected from pulses and may have missed important trade-offs. For instance, P solubilization which seems to be partially related to nitrogen fixation. Indeed complex interactions may arise when considering other properties of pulses; for example, rhizosphere acidification in response to N₂ fixation raises P availability (Xue *et al.*, 2016) and the increased phosphorus (P) availability presumably improves biomass and grain yield in intercropping (Latati *et al.*, 2016). Some components of biodiversity can also negatively impact human well-being, yet our study doesn’t consider disservices.

Finally, studies that test the effect of ecosystem structure on ecosystem services typically assume a linear relationship, an assumption that is unlikely to be widely valid as natural processes are characterized by thresholds and limiting functions (Koch *et al.*, 2009). The result is that our understanding of the relationships between ecosystem processes and provision of services remain fairly dim for most ecosystems and most services. Thus, we don’t know much about when to expect trade-offs or synergies (Bennett, Peterson and Gordon, 2009), the mechanisms that cause them, or how to minimize trade-offs and enhance synergies. Nonetheless, as in every observational method, a correlation between trait values and relative abundance does not necessarily mean a causal link between them, so such studies must be augmented with methods such as structural equation modeling that allow for more sensitive tests of causal structure (Shipley *et al.*, 2016). The PCA used to map pulses functional diversity in our study relies on linear assumption, yet agro-ecosystem properties trade-offs and trait relationships to them were studied through clustering classification and CART algorithm. Unlike logistic and linear regression, CART does not develop a prediction equation. Instead, data are partitioned along the predictor axes into subsets with homogeneous values of the dependent variable. Decision trees are a way to explore data and build predictive models. However, individual trees can be very sensitive to minor changes in the data especially when data are correlated and may need many splits before efficiently separate the variables of interest, making the interpretation more complicated.

III. Intra-specific variability

Another potential weakness of our approach is the inherent variability in some traits due to intraspecific genetic variation or phenotypic plasticity (Jung *et al.*, 2010; Kazakou *et al.*, 2014). The criticism that trait-based approaches ignore or downplay intraspecific variation and trait plasticity is still actual (Jung *et al.*, 2010; Albert *et al.*, 2011; Siefert *et al.*, 2015; Shipley *et al.*, 2016; Tautenhahn, Migliavacca and Kattge, 2020). Some existing protocols comparable to the one used in chapter 3, used for trait measurement in the field (Cornelissen *et al.*, 2003), focus on relatively few standardized individuals (mature, exposed to full light, not affected by biotic or abiotic factor) per species within one or a few environmental conditions. As a consequence, most studies in trait-based ecology use only a mean trait value per species. The original justification for this approach was that, in broad interspecific comparisons, interspecific trait variation would be much greater than intraspecific trait variation, and that existing intraspecific trait variation would not obscure the broad trends. Several studies have demonstrated that despite the significant plasticity of most plant traits across environments, the classification of species for a given trait remains fairly consistent in space and time (Garnier *et al.*, 2001; Kazakou *et al.*, 2014; Tribouillois, Fort, *et al.*, 2015). However such question have begun to be empirically studied only recently (Violle *et al.*, 2012; Jung *et al.*, 2014). The answers are still tentative and incomplete. Moreover, within those studies, traits measured on individuals of the same species are often merged in single datasets under the generic term “intra-specific variability” (Volaire, Gleason and Delzon, 2020). Consequently, genetic variation and phenotypic plasticity, as well as their interactions, are not recognized or identified, meaning that this approach cannot properly take into account the relationships between plants and the environment.

In the second chapter, due to the relatively low and uneven number of values reported across traits and species, we had to consider aggregated values for each species and each trait. After comparing different summary statistics, mean was found to be the best aggregating function (compared to median, maximum, and minimum). Standard deviation was judged non reliable to estimate the intraspecific variation, due to the relatively low and uneven number of values for evaluating intra-specific variation. However, for each documented species, trait and agro-ecosystem properties have been measured on various genotypes or cultivars, which probably accounts for within species variability. This hypothesis has made it easier to work on the initial motivating questions related to large interspecific models since, given the constraints of time and space in the experiments, a reduction in intraspecific replication facilitated the study of a wider range of species, thus offering a greater degree of generality. This was verified in the analysis of chapter 3, yet the small number of varieties used to characterize intra-species variability may have not allowed to refute this hypothesis. On the other hand, measurements showed that intra-species variability of leaf traits (SLA, LNC) was higher within than between species. This was also true for the NARA threshold.

Assuming that most functional traits have substantial levels of intraspecific variation, are highly plastic, and have values that inherently fluctuate along environmental gradients, then this would not necessarily undermine the theoretical basis of trait-based ecology; it would only mean that trait values should be measured for each individual rather than relying on average species or site values (Shipley *et al.*, 2016). Trait-based approach would then be easier to implement in crop species where varieties origin and genetics are often better described and accessible, compared with the huge genetic variation encountered in populations of spontaneous species. Application of such trait-based approach would rapidly benefit the selection of species/varieties for specific targeted agro-ecosystem services provisioning under specific (environmental or management) conditions through the understanding of how species respond to particular management techniques or environmental perturbation and how it impacts agro-ecosystem services.

IV. Trait-based approach applied to the study of diversified agro-ecosystems

Overall, this work aimed at characterizing pulses functional diversity and its relationships to agro-ecosystem properties. Main agronomic outcomes of such knowledge, apart from crop breeding strategies, would be to guide crop choice for agro-ecosystem diversification through the inclusion of pulses. The choice of crop genetic diversity may be the most important of the biodiversity choices made in agro-ecosystems (Conway, 1987). Although trait-based framework has already been largely applied to agro-ecosystems, most of this initial research focused on using traits to understand how biodiversity in agricultural systems responds to environmental conditions and land management, and concerned pollinators, weeds, or soil micro- or macro-organisms (Wood *et al.*, 2015). Research on the contribution of intercropping to productivity has largely focused on functional group classifications (Brooker *et al.*, 2015). Fewer studies have considered how functional traits influence agro-ecosystem services and could be used to design diversified agro-ecosystems.

Some constrains may come from the complexity of ecological processes in mixed-species assemblages that are not always an additive function of those in monocultures (Michel *et al.*, 2012). Until recently, no change were reported in trait-based niche differentiation in relation to change in species richness (Roscher *et al.*, 2015). However, a recent contradictory study provided a strong indication that trait plasticity might be an important driver of variability seen in experiments testing mixing effects (Dahlin *et al.*, 2020). The response of one individual cultivar can vary depending on the other cultivar in the mixture and this plastic response can significantly influence productivity in mixtures (Dahlin *et al.*, 2020). This finding can explain the inconsistent effects found in previous studies (Reiss and Drinkwater, 2018), and the difficulty of finding general “mixture effects”. Consequently, traits measured in pure stands may not be sufficient for the shape of efficient, stable and predictable cropping systems.

C. Perspectives

Trait-based approach combined with niche concept (McGill *et al.*, 2006; Wendling *et al.*, 2017) could guide the conception of appropriate mixtures of varieties and/or species to provide multiple agro-ecological services (Litrice and Violle, 2015; Martin and Isaac, 2015; Wood *et al.*, 2015; Montazeaud *et al.*, 2020). The expected relationship between traits differences and the stabilization of niche differences is the basis of many observational studies that use traits to predict patterns of species coexistence and changes in species distribution. Studies showed that trait differences generate competitive hierarchies (Kunstler *et al.*, 2012, 2016). Evidence that traits play a role in maintaining species diversity through niche partitioning comes from the great variation in trait values found within most plant communities (Westoby *et al.*, 2002) and from studies showing that trait values of co-occurring species are over-dispersed relatively to expectations (Kraft, Valencia and Ackerly, 2008; Paine *et al.*, 2011). While the literature correlating phenotypic to competitive performances has historically placed a focus on stabilization of niche differentiation, not all phenotypic differences are conducive to coexistence, and this complicates efforts to predict community assembly from trait patterns (Kraft, Godoy and Levine, 2015). Moreover, trait differences led to competitive ability differences and the dominant genotypes tended to gain more yield in mixture than the subdominant genotypes lost in yield compared to monoculture (Montazeaud *et al.*, 2018) and may bring yield benefit (Chacón-Labela *et al.*, 2019).

Niche concept have already been associated to functional trait approach in few studies. Hypervolume approaches (n -dimensional geometrical shape which can be defined by functional traits, among other things) were proposed to extend the concept of niche to functional trait diversity (Carmona *et al.*, 2016). In functional ecology, traits are often taken as proxies for niche axes (Kraft, Valencia and Ackerly, 2008; Lamanna *et al.*, 2014; Blonder, 2018). A fundamental–realized niche framework has been described by McGill *et al.* (2006). In this framework, the fundamental niche is defined as a function of plant functional traits and then serves to predict how ability to survive or reproduce changes as a result of the interaction between traits and the environment (realized niche). These perspectives have all received extensive criticism because of the weak, or generally untested, linkage between traits and niche differentiation (Kunstler *et al.*, 2012; Kraft, Godoy and Levine, 2015; Shipley *et al.*, 2016). However, many studies have highlighted that success in crop mixture is frequently driven by differences in trait expression (Montazeaud *et al.*, 2020). Competition dynamics occurring under relatively favorable conditions between genotypes are frequently driven by differences in relative growth rate and plant height which is of key importance in competition for light (Annicchiarico *et al.*, 2019). Indeed, competition for light determines the energy available for all physiological processes. Thus performance of a genotype in intercropping may depend crucially on height (Annicchiarico *et al.*, 2017), on LAI (Munz *et al.*, 2014) or on the establishment rate (Hauggaard-Nielsen and Jensen, 2001). In the same vein, under less favorable conditions (i.e.

nitrogen deficiency), traits related to better nutrient acquisition would improve the positive effect of species mixture compared to sole crops (Bedoussac and Justes, 2010b). In our study, we have identified traits related to pulses ability to rely on atmospheric nitrogen or soil cover establishment, both agro-ecosystem properties would thus be interesting for the prediction of competition dynamics.

Our study provided partial knowledge to enable the application of such framework on pulses-based diversification of cropping systems. We provided broad characterization of pulses functional diversity as well as the identification of main single traits determinant for the prediction of agro-ecosystem properties. This may allow to define a trait-based ‘niche’ which would correspond to resource acquisition strategies. In addition, we have tried to evaluate the response of nitrogen fixation to a progressive change in soil water content in relation to some leaf traits. Even if we were not able to find any relation between properties variation and leaf traits, this kind of experiment could be done more recursively to improve our understanding of agro-ecosystem reactions to environmental perturbation.

The Food and Agriculture Organization (FAO) defined “ecological intensification” (or “sustainable intensification”) within the framework of organic agriculture as “maximization of primary production per unit area without compromising the ability of the system to sustain its productive capacity”. Often, the question for alternative, sustainable cropping systems has led to adapting conventional cropping systems by prioritizing input substitution. In contrast, agrobiodiversity should better be considered as the basis to redesign sustainable agro-ecosystems by mimicking natural ecosystems (Doré *et al.*, 2011). It has been shown that farmers (empirically) use variation in crop leaf traits as an indicator to assess management prescriptions and to initiate management actions, including selection and abundance of shade tree species, crop and shade tree pruning regimes, and fertilizer treatments (Isaac *et al.*, 2018). These findings mean that functional traits represent a key nexus between scientific and local knowledge. Citizen science is a viable means to enhance functional trait data coverage (Isaac and Martin, 2019), and in doing so, can directly support theoretical and applied trait-based analyses of plants. Most cropping systems could benefit from adopting a trait-based design. The three main intercropping systems are especially suitable for plant trait-based combinations (Brooker *et al.*, 2015), as they may serve to avoid negative competition between plants with opposing traits. Based on the framework described in Gaba *et al.*, (2015) the conception of diversified cropping systems including pulses could follow a three-step process :

Step 1: The definition of the farmer’s objectives and identification of a set of services and associated agro-ecosystem properties to prioritize. Our results highlighted synergies and trade-offs between properties that can guide/ constrain the definition of farmer’s objectives compatible with pulses functional diversity. The diversification could focus on three set of properties: yield related properties, ‘cover’ related properties which have been associated to weed competitiveness in the second chapter, and nitrogen fixation ability which have been found negatively related to yield.

Step 2: Trait-based selection of species according to the targeted properties. Our results highlighted important traits for agro-ecosystem properties prediction which could guide selection of species. Traits could also permit to anticipate the variation of properties across specific management conditions or environmental perturbations.

Step 3: Improving ecosystem functions and service provision through planning and management in space and time. The type of cropping systems (i.e. rotation, intercropping, relay, break crop) as well as among soil management strategies (i.e. tillage, no-tillage) impact greatly the level of service production (Stagnari *et al.*, 2017).

Nevertheless, a better formalization of the integration of the various disciplines and definition of the notion of trait would enable a better understanding of the mechanisms underlying biodiversity variability. Modelling can make a significant contribution to cropping diversification by linking several scales (plant, plot, landscape), thus enabling diversity assessment via a functional description of species and varieties based on simple measurements of the patterns of this diversity. Moreover, diversified cropping systems can benefit from breeding programs used to develop cultivars that maximize specific architectural, morphological and physiological traits in relation to ecosystem services. Ideotypes can be developed by quantitative trait loci (QTL) detection or genomic selection methods, using markers to identify genomic areas showing highest positive interactions between genotypes or species (Gaba *et al.*, 2015).

D. References

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CHAPTER 6: *GENERAL CONCLUSION*

This thesis work provided additional knowledge for the application of trait-based framework to the diversification of cropping systems using pulses. This was achieved through a broad characterization of pulse functional trait diversity, the identification of the main determinant traits for the prediction of agro-ecosystem properties, and an analysis of physiological responses of the process underlying the relation between traits and agro-ecosystem properties to environmental change. Our results could allow to define a trait-based "niche" that would align with resource acquisition strategies and provide rules to support the choice of species and varieties to be incorporated in cropping systems for multiple services management.

Our results highlighted covariation patterns and trade-offs between agro-ecosystem properties supported by different pulses species. Our analytical framework suggests that variation in agro-ecosystem properties among pulses species follow predictions of ecological strategies theories. The main axes of pulses functional diversity were found to be driven by productive ability and competition against weed (mostly for light). This resulted in the identification of two agro-ecosystem properties 'bundle', namely 'yield and 'coverage' properties (LAI, biomass at flowering and soil cover). Nitrogen fixation ability was less representative of pulses functional diversity as it was associated to the third PCA axis but its variations were found antagonist to 'yield properties'. Our framework allowed to discuss how interactions between individual properties relate to important trade-offs among agro-ecosystem services and highlighted the importance of multi-services management instead of focusing on specific services. This work opens perspectives and provides significant insights for multi-services modeling approaches.

The description of pulses functional traits diversity highlighted leaf traits as key drivers of inter- and intra-species diversity in pulses. This result was coherent with the leaf economic spectrum, a widely described plant ecological scheme which uses leaf traits to presume for plant resource acquisition strategies. Our results highlighted contrasted leaf investment 'strategies' in relation with N and C metabolism. LNC and SLA were associated to LAR and were opposed to the overall LA indicating a trade-off between leaf area development and leaf photosynthetic capacity. On the other hand, LMR was correlated to traits indicative of growth rate indicating another trade-off between biomass investment in leaf and growth. Nitrogen content of plant organs and seed traits also captured a great part of pulses diversity. Those traits were related to agro-ecosystem properties. Although they were predicted by multiple alternative combinations of traits it was possible to identify that LNC was a major predictor of pulses agro-ecosystem properties. Other traits such as LAR, total nitrogen, seed size and cycle length were also important. The achievement of a desired set of ecosystem services may require combining antonymic or negatively correlated properties which renders the identification of suitable traits patterns more complex, as more combinations become susceptible to be suitable. Our approach was still able to highlight interesting linkages. A high LNC value together with TSW was generally associated with high yield potential. Yet a very high LNC was associated

to low yield in our experimental results and to high N fixation percentage. Investment in leaf area was important for N₂ fixation at flowering but was associated with rapid senescence and low yield. Biomass investment in leaves and a short cycle coincided with low values for almost every property except maximum soil cover. Finally, long cycles and high N content at maturity were related to delayed senescence and maintenance of N₂ fixation along pod filling.

We tried to overcome some of the criticisms made to functional trait approach. We studied trait and agro-ecosystem properties variations under different environmental conditions and within species. We compared different approaches (meta-analysis and experimental) and showed that ‘architectural’ traits (leaf or seed size) were rather stable across environments allowing to use average of heterogeneous data while trait related to resource acquisition (SLA, LNC) were less robust to environmental variations. However, SLA and LNC adjustment under progressive soil drying were found informative for pulses transpiration and nitrogen fixation regulation dynamics. Nitrogen fixation and transpiration dynamics expressed response to soil drying widely variable among accessions. The ability of *Vigna sp.* to maintain nitrogen fixation under dryer soil than chickpea was related to the average sensitivity of transpiration under drought as well as the variation in leaf trait. Even though it was not possible to draw clear correlation between leaf trait change and dynamic regulation of transpiration and N fixation, we were able to classify accessions as “water-saver” or “water-spender”. We could use such knowledge to mitigate the impact of environmental perturbation by mixing crop with various strategies.

Within-species variability as studied in chapter 3 and 4, highlighted that for some traits and processes, intra-species variability might blur the differences between species. This was clearer within species belonging to the same genus. Thus we advocate for a systematic characterization of functional diversity and its plasticity, including intraspecific variability. We argue that the trait-based approach may be easier to implement in crop species where the origin and genetics of varieties are often better described and accessible, compared to the enormous genetic variation encountered in populations of wild species. The application of this trait-based approach would rapidly benefit the selection of species/varieties for the provision of specific targeted agro-ecological services under particular (environmental or management) conditions. Yet, a better formalization of the integration of the various disciplines and definition of the notion of functional trait would enable a better understanding of the mechanisms underlying biodiversity variability. This could help defining rules for agro-ecosystem diversification. Functional traits represent a key nexus between scientific and local knowledge and can directly support theoretical and applied trait-based analyses of crop and agro-ecosystem design.

ANNEXES

ANNEXES 1: COMPLETE LIST OF REFERENCES USED TO BUILD THE DATABASE

Auteurs	Titre	Date
Bolaji U. Olayinka, Emmanuel O. Etejere	Growth analysis and yield of two varieties of groundnut (<i>Arachis hypogaea</i> L.) as influenced by different weed control methods	2015
Rainer Gross	El cultivo y la utilización del tarwi: <i>Lupinus mutabilis</i> Sweet	1982
Wilson Urrutia Gutiérrez	Determinación de parámetros óptimos de extracción alcalina para la obtención de aislado proteico a partir de tarwi (<i>lupinus mutabilis</i>)	2012
James A. Duke	Handbook of Energy Crops.	1983
K.J. Boote	Growth Stages of Peanut (<i>Arachis hypogaea</i> L.)	1962
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Aziza Zoghalmi, Mongi Zouaghi	Morphological variation in <i>Astragalus hamosus</i> L. and <i>Coronilla scorpioides</i> L. populations of Tunisia	2003
Jaime Prohens, Isabel Andújar, Santiago Vilanova, Mariola Plazas, Pietro Gramazio, Rafael Prohens, Francisco J. Herraiz, Antonio M. De Ron	Swedish coffee (<i>Astragalus boeticus</i> L.), a neglected coffee substitute with a past and a potential future	2014
Nasir El Bassam	Handbook of bioenergy crops : a complete reference to species, development and applications	2010
Hector Valenzuela, Jody Smith	Green Manure Crops: Lablab	2002
M.J. du Plessis, D. Fourie, A.J. Liebenberg, M.M. Liebenberg, C.J. van Zyl	Dry bean	2001
J. Smartt	The groundnut crop; a scientific basis for improvement.	2012
Tendai P. Chibarabada, Albert T. Modi, Tafadzwanashe Mabhaudhi	Expounding the value of grain legumes in the semi- and arid tropics	2017
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H. Zhang, M. Pala, T. Oweis, H. Harris	Water use and water-use efficiency of chickpea and lentil in a Mediterranean environment	2000
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Y. A. Abayomi, T. V. Ajibade, O. F. Sammuell, B. F. Sa'adudeen	Growth and yield responses of cowpea (<i>Vigna unguiculata</i> (L.) Walp) genotypes to nitrogen fertilizer (NPK) application in the Southern Guinea Savanna zone of Nigeria	2008
L Vimalendran, K R Latha	Yield, water use and water use efficiency of pigeonpea [<i>Cajanus cajan</i> (L.) Millsp.] under drip fertigation system	2014
K. H.M. Siddique, K. L. Regan, D. Tennant, B. D. Thomson	Water use and water use efficiency of cool season grain legumes in low rainfall Mediterranean-type environments	2001
P. Geil, J. Anderson	Nutrition and health implications of dry beans: a review.	1994
V. N. Atasie, T. F. Akinhanmi, C. C. Ojiodu	Proximate analysis and physico-chemical properties of groundnut (<i>Arachis hypogaea</i> L.)	2009

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Patti Bazel Geil, James W Anderson	Nutritional quality of important food legumes	2006
KeShun Liu	Soybeans : Chemistry and Nutritional Value of Soybean Components	1997
R. K. Deka, C. R. Sarkar	Nutrient composition and antinutritional factors of Dolichos lablab L. seeds	1990
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G. Bejiga, L.J.G. van der Maesen	Cicer arietinum L. [Internet] Record from PROTA4U	2006
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V. Heuzé ,H. Thiollet , G. Tran , R. Delagarde , D. Bastianelli , F. Lebas	<i>Pigeon pea (Cajanus cajan) forage</i>	2017
Florence Tardy, Delphine Moreau, Marc Dorel, Gaëlle Damour	Trait-based characterisation of cover plants' light competition strategies for weed control in banana cropping systems in the French West Indies	2015
V Tomar S Singh G Tripathi S	Crop weed competitions in arhar (<i>Cajanus cajan</i> L.) under northern west plain zone	2003
A. R. Sheldrake, A. Narayanan	Growth, development and nutrient uptake in pigeonpeas (<i>Cajanus cajan</i>)	1979
L Vimalendran, K R Latha,	Yield, water use and water use efficiency of pigeonpea [<i>Cajanus cajan</i> (L.) Millsp.] under drip fertigation system	2014

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Remmy Raphael Mwakimbwala	COMPARATIVE IMPROVEMENT IN SOIL NITROGEN BY FIVE LEGUMINOUS COVER CROPS AT UYOLE AGRICULTURAL RESEARCH INSTITUTE, MBEYA, TANZANIA	2015
K. Balakrishnan, N. Natarajaratnam, C. Rajendran	Critical Leaf Area Index in Pigeonpea	2008
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Ranjeet Patel, Singh RKR, Varun Tyagi, Mallesha, Raju PS	Nutritional evaluation of <i>Canavalia ensiformis</i> (Jack bean) cultivated in North East region of India	2016
Suriyan Cha-um, Charlie B. Batin, Thapanee Samphumphung, Chalernpol Kidmanee	Physio-morphological changes of cowpea (<i>vigna unguiculata walp.</i>) And jack bean (<i>canavalia ensiformis</i> (L.) DC.) In responses to soil salinity	2013
Virupax C. Baligar, Marshall Elson, Zhenli L. He, Yuncong Li, Arlicelio de Q. Paiva, Dario Ahnert, Alex-Alan F. Almeida and Nand K. Fageria	Ambient and Elevated Carbon Dioxide on Growth, Physiological and Nutrient Uptake Parameters of Perennial Leguminous Cover Crops under Low Light Intensities	2015
C. D. J. Kessler	An Agronomic Evaluation of Jackbean (<i>Canavalia ensiformis</i>) in Yucatan, Mexico. I. Plant Density	1990
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Piara Singh, S.M. Virmani	Modeling growth and yield of chickpea (<i>Cicer arietinum</i> L.)	1996
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Ranjeet Kour, B.C. Sharma, Anil Kumar, Paramjeet Kour, Brij Nandan	Study of physiological growth indices of mustard in chickpea (<i>cicer arietinum</i>)+ mustard (<i>brassica juncea</i>) intercropping system under different weed management practices	2016
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MUHAMMAD ARSHAD, A. BAKHSH, ABDUL GHAFOOR	PATH COEFFICIENT ANALYSIS IN CHICKPEA (CICER ARIETINUM L.) UNDER RAINFED CONDITIONS	2004
D.F. Beech, H. Stutzel, D.A. Charles-Edwards	Yield determinants of Guar (Cyamopsis tetragonoloba): 1. Grain yield and pod number	1989
A. R. Mubarak, N. O. Salih and A. A. Hassabo	Fate of 15N-labeled urea under a guar-wheat rotation as influenced by crop residue incorporation in a semi-arid Vertisol.	2016
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R. S. Yadav	Effects of Weed Removal in Clusterbean (Cyamopsis tetragonoloba) under Different Rainfall Situations in an Arid Region	1998
B.S. AFRIA, N.S. NATHAWAT, M.L YADAV,	Effect of cycocel and saline irrigation of physiological attributes, yield and its components in different - varieties of guar (cyamopsis tetragonoloba l . Taub .)	1998
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R.C.Muchow	Canopy development in grain legumes grown under different soil water regimes in a semi-arid tropical environment	1985
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ABSTRACT

Pulses are generally recognized for their multiple nutritional and environmental benefits. However, most legume species can be defined as orphan crops. This underlines the lack of knowledge on legume diversity and the importance of studying this diversity and elucidating how it supports the functioning of agro-ecosystems. Functional trait approach could guide the inclusion of pulses into agro-ecosystems to provide one or more services. This work aimed at synthesizing current knowledge on the functional diversity of cultivated pulses at the interspecific level as well as producing new insights on the relationships between pulses functional trait variation and agro-ecosystems functioning. We focused on agro-ecosystem properties hypothesized to be good indicators of three specific agro-ecosystem services related to food provision in low-input systems: yield under dry conditions, nitrogen (N) fixation, and competitiveness toward weeds. We combined concepts and methods from functional ecology, crop physiology and agronomy. We applied multivariate statistical methods to data collected from the literature and to data collected from an original field experiment to explore traits-agro-ecosystem properties relationships under multiple environments (literature data) or optimal environmental conditions (field experiment). A controlled experiment in pots allowed to assess the effect of water deficit on key processes such as transpiration and biological N fixation. Our results highlighted covariation patterns and trade-offs between agro-ecological properties supported by different pulses species. Pulses diversity was also described through functional traits and indicated leaf traits as key drivers of inter- and intra-species variability. Nitrogen content of plant organ and seeds traits also captured a great part of pulses diversity. Those traits were related to agro-ecosystem properties. Although they were predicted by multiple alternative combinations of traits it was possible to identify that leaf N content was an important predictor of pulses agro-ecosystem properties. We compared different approaches (meta-analysis and experimental) and discussed intra-species variability. Controlled experiment revealed a large intra and interspecific variability in the response of N fixation and transpiration dynamics, as well as relationships between leaf traits, C/N metabolism, and soil drying-responses of growth, transpiration, and N₂ fixation. We also discussed the intrinsic limitations of trait-based approach and pertinence for its application to agro-ecosystem diversification.

RESUME

Les légumineuses à graines sont généralement reconnues pour leurs nombreux avantages nutritionnel et environnementaux. Cependant, la plupart de ces espèces peuvent être définies comme des cultures orphelines. Ce constat souligne le manque de données et de connaissances quant à la diversité des légumineuses. L'étude de cette diversité via l'approche des traits fonctionnels pourrait guider l'insertion des légumineuses dans les agro-écosystèmes. Le but de ces travaux a été de synthétiser les connaissances actuelles sur la diversité fonctionnelle des légumineuses cultivées au niveau interspécifique, ainsi que de produire de nouvelles connaissances sur les relations entre les traits fonctionnels et le fonctionnement des agro-écosystèmes. Nous nous sommes concentrés sur un certain nombre de propriétés des agro-écosystèmes dont nous supposons qu'elles sont de bons indicateurs de trois services agroécosystemiques importants et liés à l'approvisionnement alimentaire des système bas intrants : le rendement en conditions sèches, la fixation de l'azote et la compétitivité face aux adventices. Pour cela, nous avons combiné des méthodes et des approches issues de différentes disciplines : écologie fonctionnelle, physiologie et agronomie. Nous avons appliqué des méthodes de statistique multivariée sur une base de données collectées dans la littérature puis aux données originales recueillies lors d'une expérience au champ pour explorer les relations entre traits et propriétés des agro-écosystèmes dans des environnements multiples (données de la littérature) ou dans des conditions environnementales communes et optimales (expérience sur le terrain). Une expérience en conditions contrôlées a également permis d'évaluer l'effet du déficit en eau sur des processus clés tels que la transpiration et la fixation biologique de l'azote. Nos résultats ont mis en évidence la structure de covariation ainsi que des compromis entre les propriétés soutenues par différentes espèces de légumineuses. La diversité des légumineuses a également été décrite par le biais de traits fonctionnels. Les traits foliaires ont été mis en évidence comme étant le principal élément de variabilité inter- et intra-espèces. La teneur en azote des organes végétaux et des semences constituait également un facteur important de la diversité des légumineuses. Ces traits ont été liés aux propriétés des agro-écosystèmes. Bien que ces derniers puissent être prédits par de multiples combinaisons de traits, il a été possible d'identifier le LNC comme un indicateur important des propriétés de l'agro-écosystème. Nous avons comparé différentes approches (méta-analyse et expérimentale) et discuté de la variabilité intra-espèce. L'expérience contrôlée a révélé une grande variabilité intra et interspécifique dans la réponse de la fixation de l'azote et de la dynamique de la transpiration, ainsi que des relations entre traits foliaires, métabolisme C/N et réponses de la croissance, de la transpiration et de la fixation du N₂ au dessèchement du sol. Nous avons également discuté des limites intrinsèques de l'approche basée sur les caractères et de son potentiel pour l'application dans la conception d'agro-écosystèmes diversifiés.