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Analyse des processus de compétition et de facilitation dans les agrosystèmes canne-à-sucre x légumineuses.

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Résumé

Pour réduire l'usage d'herbicide et d'engrais azoté les acteurs de la filière canne à sucre expérimentent depuis plusieurs années des associations avec des légumineuses. Cependant, l'introduction d'une nouvelle plante complexifie le système de culture et peut représenter un défi pour concilier les services attendus (contrôle des adventices et enrichissement du système en azote) avec les performances agronomiques (risque de compétition interspécifique excessive). Ainsi, l'objectif général de la thèse était de caractériser les interactions de compétition et de facilitation entre canne à sucre et légumineuse. Ce travail repose d'une part, sur une méta-analyse couplant, de manière originale, des données de la littérature scientifique et des données issues d'un réseau d'essais mené à La Réunion et d'autre part, sur un essai expérimental représentant un cas d'étude : canne à sucre en association avec Canavalia ensiformis (L.) DC.,1825 - Pois sabre blanc.

Les résultats ont montré que les légumineuses ont eu un impact négatif plus faible sur le rendement de la canne à sucre que les adventices, renforçant l'intérêt des légumineuses pour réduire l'utilisation des herbicides. Cependant, les légumineuses entrainaient une baisse de rendement de canne de 3%, en moyenne, mais avec des variations importantes allant de -65% à +47%. Les dates de semis et de destruction de la légumineuse sont des facteurs clés pour orienter et moduler la compétition entre les espèces. Dans notre cas d'étude, la présence de légumineuses a affecté négativement la nutrition azotée de la canne, entraînant une réduction de la biomasse aérienne, racinaire et du tallage. Cet impact sur la disponibilité en ressources du sol semble être indirect via une diminution du volume de sol accessible par les racines de canne en présence de légumineuse. La compétition entre les espèces était fortement influencée par la disponibilité des ressources, en particulier l'eau, qui a eu un impact direct sur l'offre en azote du sol, soulignant l'importance de la gestion de l'irrigation dans ces systèmes.

Les données de la méta-analyse ont montré une augmentation du rendement en canne à sucre dans environ un tiers des cas, mettant en évidence une facilitation des légumineuses envers la canne à sucre. Toutefois, dans notre cas d'étude, les pertes d'azote du système sol-plante lors de la décomposition des résidus de légumineuse ont été équivalentes à la quantité d'azote apportée par la fixation de ces légumineuses. Ce résultat découle en partie des choix visant à promouvoir la croissance de la canne à sucre aux dépens de celle des légumineuses, ainsi que des conditions favorables à la volatilisation de l'azote (résidus laissés en surface, décomposition rapide des résidus, conditions climatiques et présence du mulch de canne).

Ainsi, cette thèse met en évidence les limites de la gestion actuelle des légumineuses dans les systèmes canniers de La Réunion, contribue à enrichir notre compréhension des interactions multi-spécifiques dans ces systèmes, et offre ainsi des indications sur les pratiques culturales à adopter pour optimiser les services rendus par les légumineuses.

Mots-clés : Association de culture ; Plante de services ; Plante compagne ; Fabaceae ; *Saccharum officinarum*

Abstract

To reduce herbicide and nitrogen fertilizer use, sugarcane legume intercropping sugarcane has been experimented for several years. However, introducing a new plant complicates the cropping system. It can represent a challenge in reconciling the expected services (weed control and nitrogen enrichment) with agronomic performance (risk of excessive interspecific competition). Therefore, the thesis aimed to characterize the competitive and facilitative interactions between sugarcane and legumes. This work is based, on the one hand, on a meta-analysis combining, in an original way, data from the scientific literature and data from a network of trials carried out in La Réunion and, on the other hand, on an experimental trial representing a case study: sugarcane in association with Canavalia ensiformis (L.) DC.,1825 – Jack Bean.

The results showed that legumes had a smaller negative impact on sugarcane yield than weeds, reinforcing the interest in legumes in reducing herbicide use. However, on average, legumes reduced sugarcane yield by 3%, with significant variations ranging from -65% to +47%. Legume sowing and destruction dates are key factors in determining and modulating competition between species. In our case study, the presence of legumes negatively affected the nitrogen nutrition of the sugarcane, leading to a reduction in above-ground biomass, root biomass, and tillering. This impact on soil resource availability appears to be indirect via reducing the volume of soil colonized by sugarcane roots in the presence of legumes. Competition between species was strongly influenced by the availability of resources, particularly water, which directly impacted soil nitrogen supply, underlining the importance of irrigation management in these systems.

Data from the meta-analysis showed an increase in sugarcane yield in around a third of cases, highlighting a facilitation of legumes towards sugarcane. However, in our case study, nitrogen losses from the soil-plant system during the decomposition of legume residues were equivalent to the amount of nitrogen provided by legume fixation. This result stems partly from choices made to promote sugarcane growth at the expense of legume growth and from conditions favorable to nitrogen volatilization (residues left on the surface, rapid residue decomposition, climatic conditions, and the presence of sugarcane mulch).

This thesis highlights the limits of current legume management in sugarcane systems on Reunion Island, contributes to our understanding of multi-species interactions in these systems, and offers indications as to how to adapt cultural practices to optimize the services rendered by legumes.

Keywords: Intercropping; Cover crop; Companion plant; Fabaceae; *Saccharum officinarum*

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Introduction générale

1. Préambule

La **canne-à-sucre** joue un rôle significatif dans l'économie mondiale, tant dans le secteur de l'agro-alimentaire que dans celui de l'énergie. Cette filière, comme de nombreuses autres, cherche à réduire ses impacts négatifs sur l'environnement en faisant évoluer les itinéraires techniques au champ.

La **durabilité** de la filière canne-à-sucre à La Réunion relève d'une importance économique, sociétale et environnementale. L'activité industrielle de la canne-à-sucre est conditionnée par un tonnage critique de canne usinable actuellement atteint. Dans un contexte insulaire marqué par l'impossibilité d'étendre les terres cultivables et une pression urbaine forte, la production en baisse depuis plusieurs années dans l'île fait planer une menace sur la pérennité de la filière canne-à-sucre (Agreste, 2023). Parmi les préoccupations les plus pressantes des planteurs figurent la disparition de molécules homologuées pour lutter contre les adventices, la manque de main-d'œuvre et l'explosion du prix des engrais et de divers intrants, aggravée par celle du fret maritime (Le Figaro, 2023).

Face au double objectif de substitution des intrants de synthèse et de réduction des impacts environnementaux, les acteurs de la filière testent depuis plusieurs années l'introduction de plantes de services, telles que les **légumineuses**, dans le système de culture. Or, si l'efficacité des légumineuses dans le contrôle des adventices est avérée (Mansuy et al., 2019), l'effet sur le rendement en canne est lui très variable.

Cette thèse a été financée par le Cirad et l'institut de recherche technique eRcane afin de répondre aux besoins de la filière canne en la matière. Les travaux de recherche ont été menés à La Réunion au sein des unités de recherche Aida et Recyclage et Risque du Cirad basés à la station de la Bretagne à Saint-Denis.

Le sujet de la thèse s'inscrit dans un objectif général d'approfondissement des connaissances sur les **interactions** (compétition et/ou facilitation) entre **canne-à-sucre et légumineuse en associations** pour les ressources du milieu (lumière, eau et azote), ainsi que d'évaluation de l'impact de la légumineuse de service sur la **fertilité** des agrosystèmes.

Ce mémoire débute par la présentation du contexte de l'étude et des connaissances utiles à la compréhension des associations de cultures entre canne et légumineuse. Après la présentation de la problématique de la thèse et de la démarche adoptée, le travail de recherche se décline ensuite en trois chapitres : 1/ une synthèse des connaissances actuelles sur l'effet des associations sur le rendement de la canne-à-sucre à travers une méta-analyse, 2/ l'évaluation des processus de compétition entre canne et légumineuse dans un cas de référence en station expérimentale et, 3/ l'évaluation du devenir de l'azote issu de la fixation biologique dans cette association de référence. Pour finir, une conclusion générale intégrera les différentes perspectives scientifiques des résultats de la thèse.

2. L’agro-écosystème cannier

2.1. La canne-à-sucre : des enjeux globaux

Histoire millénaire de l’herbe géante gorgée de sucre

La canne-à-sucre (*Saccharum officinarum L.*) est une grande graminée vivace cultivée depuis la préhistoire pour le saccharose qu’elle accumule dans ses tiges. Originaire de Papouasie-Nouvelle-Guinée, elle est l'une des premières plantes à avoir été domestiquées vers 10 000 avant JC à partir de l’espèce sauvage *S. robustum* (Martignac, 2006). L’extraction et l’exploitation du sucre semble avoir commencé en Inde vers 3000 ans avant JC (Meyer, 1989). Par la suite, elle a voyagé au fil des siècles à travers l'Asie, l'Afrique, le Moyen-Orient, l’Europe, et les Amériques devenant une culture emblématique pour de nombreuses civilisations.

Le rôle économique majeur de la canne-à-Sucre

Aujourd’hui, la canne est la première culture cultivée au monde en termes de quantité produite (Faostat, 2021). Elle représente près de 23% de la production agricole mondiale avec une production annuelle de sucre qui atteint 180 millions de tonnes, provenant à la fois d'industries et de producteurs indépendants de toutes tailles (CIRAD, 2022; Terre-net, 2019). Présente dans un peu plus de 100 pays, la canne-à-sucre a un rôle économique et culturel dans de nombreux pays des régions tropicales et subtropicales (Moore et al., 2013). Les principaux pays producteurs sont le Brésil, l'Inde et la Chine (FAO, 2014). En France, la culture de canne-à-sucre est répartie dans 2 régions d’outre-mer : la Guadeloupe et la Martinique aux Antilles, et la Réunion dans l’océan Indien. La transformation de ses tiges est au cœur de son exploitation, permettant la production de sucre et de rhum, mais aussi la production bioéthanols de première et seconde génération, d’électricité à travers la combustion de la bagasse et de matières premières pour l'industrie chimique à partir de la mélasse.

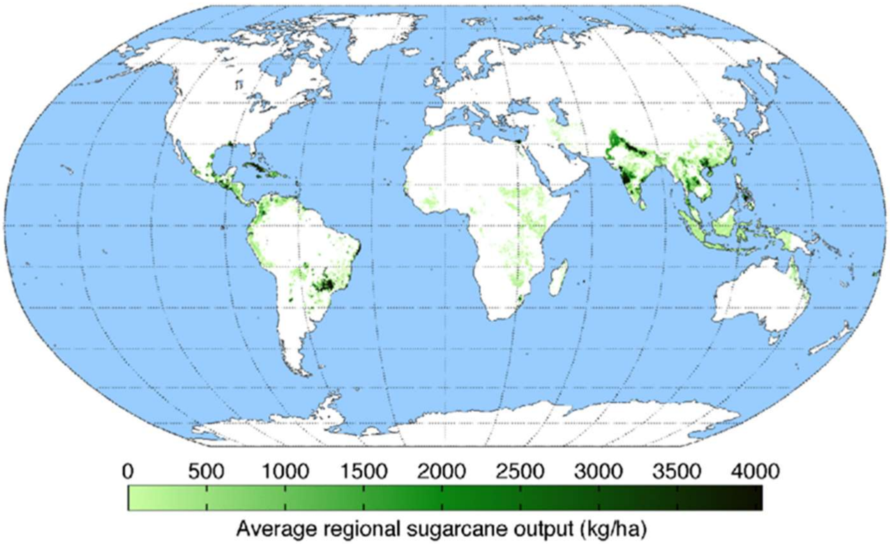

Figure 1. Surface de production de canne dans le monde. Source : Scortecci et al. (2012)

Une demande mondiale croissante

La canne-à-sucre couvre 80% de la production totale de sucre, le reste étant fourni par la betterave sucrière (Terre-net, 2019). La demande mondiale en sucre ne cesse de croître, stimulée par la croissance démographique et l'évolution des habitudes alimentaires (OCDE, 2022). Outre cette prépondérance dans le domaine sucrier, elle assume un rôle grandissant dans la production d'énergies renouvelables, à une époque où la demande en énergie verte se fait de plus en plus pressante. Depuis les années 1970, le Brésil a développé une importante filière de bioéthanol destiné à être utilisé comme agro-carburant (Stolf and Oliveira, 2020). Aujourd'hui, plus de 50% de la production brésilienne de canne-à-sucre est destinée à la production d'éthanol carburant (OCDE, 2022). La coexistence de la production de sucre et d'énergie a engendré un déficit de 15 millions de tonnes de sucre au cours de la dernière décennie, comblée grâce aux réserves mondiale (OCDE, 2022). Compte tenu de la croissance démographique, la demande mondiale en sucre de canne pourrait atteindre 210 millions de tonnes d'ici 2030 (CIRAD, 2022).

L'équilibre entre productivité et durabilité

L'augmentation de la demande conduit à l'expansion des terres cultivées, ce qui comporte le risque de fragmenter les paysages naturels tels que les forêts et les savanes, mettant ainsi en péril la biodiversité (Picoli and Machado, 2021). Pour assurer la durabilité de la culture de la canne-à-sucre, il est essentiel de relever le défi de la croissance de la demande tout en réduisant au maximum les impacts environnementaux. Cette démarche englobe le développement de pratiques agricoles durables, la recherche de variétés de canne plus performantes, ainsi que la gestion responsable des terres dans le but de préserver la biodiversité et de minimiser l'empreinte environnementale de cette culture polyvalente (CIRAD, 2022).

2.2. La canne-à-sucre au centre de l'agriculture réunionnaise

Culture emblématique de l'île de La Réunion

La canne-à-sucre imprègne l'histoire de la Réunion autant que sa géographie et sa culture (Marimoutou et al., 2021; Martignac, 2006). Implantée sur l'île dès le 17ème siècle par les colons français, la canne-à-sucre est d'abord cultivée pour fabriquer de l'alcool. Sa production ne devient une véritable industrie qu'au début du 19ème, supplantant notamment la culture du café qui avait fait un temps la richesse de l'île. L'île comptait plus de 200 usines sucrières à son apogée, dont seules deux subsistent aujourd'hui, celles du Gol à Saint-Louis et de Bois Rouge à Sainte-Suzanne. Aujourd'hui, la canne-à-sucre demeure toujours la culture prédominante de l'île, couvrant 54% de la surface agricole utilisée, soit environ 21 000 hectares (Figure 2 ; Agreste, 2023).

Un pilier économique de l'île

La filière Canne-Sucre joue un rôle central sur le plan économique et social, générant environ 18 300 emplois directs, indirects et induits, ce qui équivaut à environ 13% des emplois du secteur privé de l'île (DAAF, 2022a). La transformation de la canne-à-sucre est supervisée par le groupe français Tereos Océan Indien au sein des deux sucreries de l'île. Chaque année, environ 1,6 million de tonnes de cannes sont récoltées, produisant en moyenne 183 000 tonnes de sucre (Agreste, 2023). La moitié de cette production est destinée au marché des sucres spéciaux (blonds, roux, bruns, foncés, etc.), où La Réunion occupe une position de leader en Europe, tandis que l'autre moitié est destinée à être raffiné (Agreste, 2023). La filière Canne-Sucre exporte plus 90% de sa production totale vers l'Europe, ce qui la positionne en tête des exportations de l'île, contribuant à hauteur de 80% en volume et d'environ 50% en valeur (Agreste, 2023).

Une culture multifonctionnelle

La filière valorise 100% des sous-produits issus des usines sucrières (bagasse, mélasse et écume). La mélasse est majoritairement valorisée pour la production de rhum dans les quatre distilleries de l'île. Les rhums et spiritueux à base de rhum sont le troisième produit le plus exporté de La Réunion avec environ 21 millions d'euros de revenus à l'exportation (Agreste, 2023).

La bagasse, issue du processus d'extraction du sucre, est exploitée à des fins énergétiques. Les deux centrales thermiques mixtes bagasse-charbon, situées à proximité des usines sucrières, convertissent 540 000 tonnes de bagasse en énergie, ce qui permet d'éviter l'importation et la combustion de 138 000 tonnes de charbon chaque année (Tereos Océan Indien, s.d.). Cela représente environ 10% des besoins énergétiques de l'île de contribuant ainsi ses objectifs d'autonomie énergétique (DAAF, 2022a).

Enfin, il existe de nombreuses synergies entre la filière canne-à-sucre et les autres secteurs agricoles (Figure 2). Une partie de la mélasse est utilisée comme complément alimentaire pour le bétail, et les écumes et les cendres de bagasse sont revalorisée comme engrais organiques pour les cultures. La paille de canne permet de couvrir plus de 30% des besoins en fourrage et en litière de l'île (Tereos Ocean Indien, s.d.). La paille est également utilisée en paillis pour les cultures maraîchères. De plus, les champs de canne offrent une solution pour recycler et valoriser les effluents d'élevage produits localement.

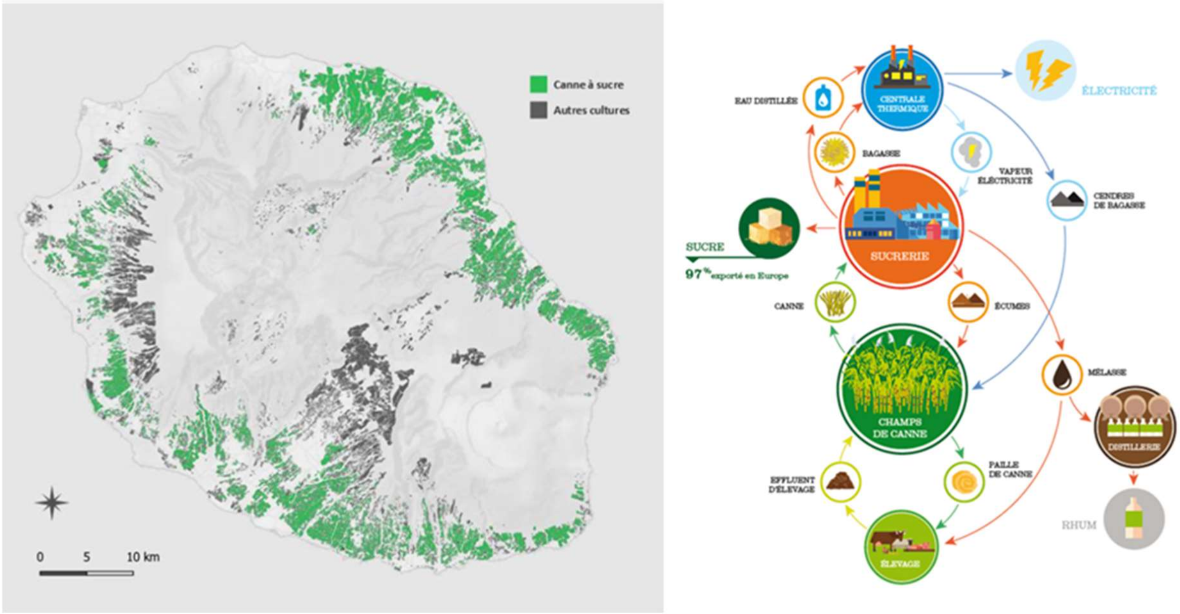

Figure 2. Surfaces agricoles utiles cultivées sous canne-à-sucre à La Réunion (selon la base d'occupation des sols de 2019, source : Ramos, 2023) et devenir des co-produits de la canne à La Réunion (source : Tereos Océan Indien, s.d.).

2.3. La culture de la canne-à-sucre

Morphologie et phénologie de la canne-à-sucre

La canne-à-sucre est une plante vivace de la famille des Poaceae ressemblant au roseau. Un plant de canne est une touffe de 5 à 20 tiges, appelées "talles", mesurant jusqu'à 5 mètres de hauteur (Figure 3). Chaque tige se compose d'une succession de nœuds, abritant un bourgeon, un anneau d'ébauches de racines et sur lesquelles s'insèrent des feuilles ainsi que des entre-nœuds qui stockent le sucre produit par la plante. L'inflorescence est une panicule terminale de grande taille composée d'une multitude de fleurs.

La multiplication de la canne-à-sucre pour l'agriculture se fait par voie végétative en utilisant des boutures composées généralement de 1 à 4 bourgeons. Les morceaux de tige sont mis en terre en ligne, dont l'espacement varie selon les pratiques spécifiques à chaque région (généralement 1.5 m à La Réunion) (Poser, 2013).

De la plantation à la récolte, le cycle de croissance de la canne-à-sucre comporte quatre étapes principales : la germination et l'enracinement des bourgeons, la levée et le début du tallage, la croissance végétative, et enfin la maturation caractérisée par l'accumulation de sucre dans les tiges (Figure 4).

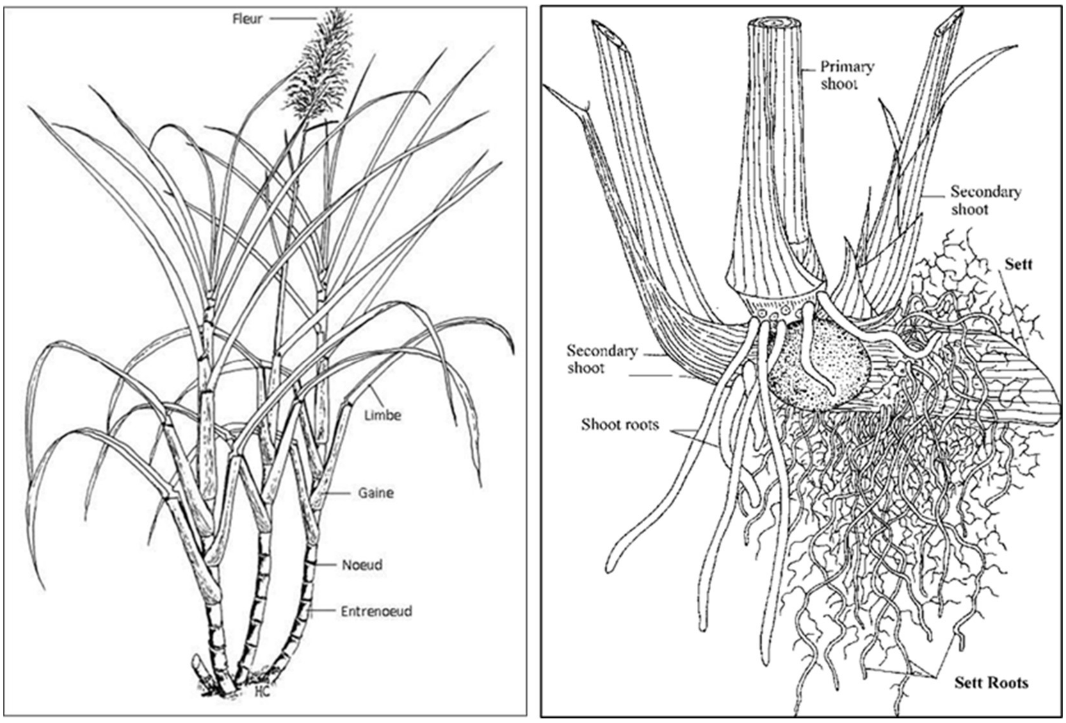

Figure 3. Schéma botanique de la canne-à-sucre (gauche, source : Soopramanien, 2000) et système racinaire d'une jeune canne en plantation (droite, source : Blackburn, 1984).

La phase de croissance végétative de la canne-à-sucre s'étend sur les 6 premiers mois du cycle, jusqu'à la fermeture de la canopée. Cette phase a lieu pendant la saison des pluies à La Réunion, de décembre à avril. Pendant les premiers stades de l'installation du couvert, la canne est particulièrement sensible à la température, à la sécheresse et à l'intensité du rayonnement solaire (Bezuidenhout et al., 2003; Singels et al., 2005). Ensuite, la phase de maturation, d'environ 5 à 6 mois, survient pendant la saison sèche et fraîche (de mai à novembre). La réduction des températures et des précipitations favorise l'accumulation de sucre dans les tiges au détriment de la biomasse fibreuse, grâce aux stress hydriques et/ou thermiques subit par la canne.

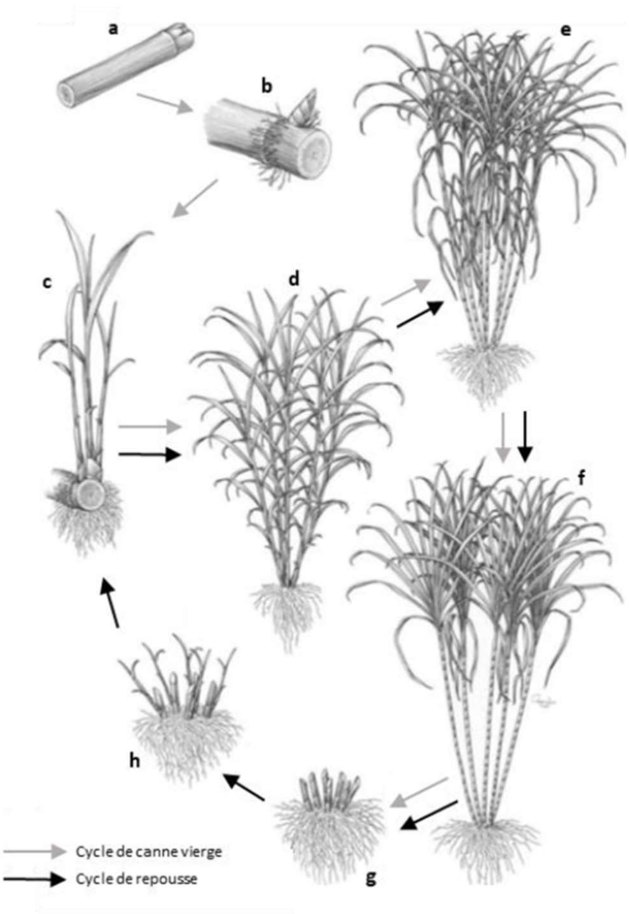

Figure 4. Cycle phénologique et cultural de la canne-à-sucre représentés par la bouture (a), le débourrement des bourgeons et le développement racinaire (b), le tallage (c), la croissance végétale (d), la maturation (e), la maturité (f), la récolte (g), la repousse des talles (h) selon Cheavegatti-Gianotto et al. (2011).

Le cycle de culture de la canne-à-sucre

La culture de la canne-à-sucre est dite semi-pérenne. La plantation marque le début du cycle de culture avec un premier cycle de canne "vierge" (plant crop en anglais). Lors de la récolte, la tige est coupée au ras du sol. La souche donnera ensuite naissance à de nouvelles talles, correspondant à une nouvelle production ou "repousse" (ratoon en anglais). Le cycle de récolte de la canne est généralement annuel. À La Réunion, la "campagne sucrière" débute vers le mois de juin au début de la saison sèche et se conclut au mois de décembre au début de la saison des pluies.

Au cours des repousses successives, le rendement de la parcelle tend à diminuer. La parcelle est alors labourée et de nouvelles plantations sont mises en terre (nouveau cycle cultural) généralement après une période de jachère de 2 à 4 mois. Le nombre de repousses dépend de la productivité de la parcelle (Pouzet, 2011) et de sa facilité d'accès et du degré de mécanisation. Le CTICS (Centre Technique Interprofessionnel de la Canne et du Sucre) de l'île de La Réunion recommande un intervalle de 7 ans entre chaque plantation. Cependant, dans la plupart des exploitations agricoles de l'île, la période moyenne qui s'écoule entre chaque nouvelle plantation est d'environ 10 ans (Chabalier et al., 2006).

Systèmes de culture de la canne à la Réunion

La filière repose sur un modèle social composé d'exploitations familiales de petite dimension (71 % des planteurs cultivent moins de 8 hectares) (DAAF, 2022b). Environ 3000 planteurs consacrent tout ou partie de leurs activités à la culture de la canne-à-sucre, dont la plupart récolte encore la canne manuellement (Agreste, 2023). Le paysage agricole est ainsi très découpé. Les champs de canne sont répartis sur les pentes de l'île, entre 0 et 800 m d'altitude.

La culture est conduite en conditions pluviales à l'Est de l'île, où les précipitations peuvent atteindre jusqu'à 11 000 mm/an et en conditions irriguées à l'Ouest, où les précipitations sont bien inférieures avec 450 mm/an (Darras et al., 2021). La canne est majoritairement cultivée en monoculture, son cycle pluriannuel limitant la rotation, avec beaucoup d'intrants (herbicides et engrais minéraux). Les cultures intercalaires, traditionnellement utilisées pour une production alimentaire secondaire, se sont marginalisées au cours du siècle dernier.

Les variétés cultivées proviennent de la création variétale locale assurée par le centre de recherche eRcane depuis 90 ans et sont adaptées aux contraintes climatiques et aux objectifs de production des planteurs (production de biomasse et de fibres et richesse en sucre) (eRcane, s.d.). Les rendements (70 à 140 t ha^{-1}) sont très hétérogènes sur l'île en raison de la forte diversité agronomique, pédo-climatique et technique des zones de production (DAAF, 2022a).

2.4. Les défis de la filière canne à la Réunion

Un impératif de production

La rentabilité des usines sucrières est conditionnée par un seuil minimum de tonnage de canne usinable actuellement atteint. Au cours de la dernière décennie, les terres consacrées à la culture de la canne-à-sucre ont connu un recul important, avec une perte de superficie d'environ 3 000 hectares, traduisant les effets combinés de la diversification des cultures (prairies) et de la pression de l'urbanisation (Agreste, 2023). Dans ce contexte insulaire marqué par l'impossibilité d'étendre les terres cultivables, la production en baisse depuis plusieurs années fait planer une menace sur la filière canne-sucre. Ainsi, le maintien voire l'augmentation de la productivité à l'échelle des parcelles est donc une nécessité pour l'avenir de la canne à La Réunion dans le modèle économique actuel.

Une culture soumise à une forte pression des adventices

Grâce à un processus de sélection rigoureux, les variétés cultivées à La Réunion sont tolérantes aux maladies et aux insectes. Ainsi en termes de bioagresseurs c'est la gestion des adventices au sein des exploitations cannières qui reste le principal défi des planteurs. Le climat tropical de l'île, caractérisé par une absence de saison froide marquée et des précipitations très élevées, crée un environnement propice à la croissance continue et rapide des adventices. La canne-à-sucre est principalement sensible à la présence d'adventices dans ses premiers mois de croissance (Antoir et al., 2016; Cheavegatti-Gianotto et al., 2011). Au-delà de quatre mois de cycle, la levée de nouvelles adventices n'a que peu d'impact sur la croissance et le développement de la canne, la fermeture de la canopée limitant largement le développement des adventices (Antoir et al., 2016; Martin et al., 2012). Les lianes grimpantes restent néanmoins problématiques grâce à leur capacité à s'ériger au-delà du couvert de canne, cet accès à la lumière leur permettant ensuite de se développer, de recouvrir la culture et d'in fine impacter significatif sur le rendement (Martin et al., 2010; Ponsart, 2022).

La disparition des herbicides

L'utilisation d'herbicides est le moyen de lutte privilégié contre les adventices par les planteurs à l'heure actuelle, ce qui fait de la canne-à-sucre, par son emprise sur la SAU de l'île, une culture très consommatrice d'herbicides, malgré un IFT relativement bas (3,4) par rapport à celui d'autres cultures (Agreste, 2016). Le retrait du marché de plusieurs herbicides, notamment de l'asulame à la fin de 2012 (seule molécule herbicide sélective de la canne-à-sucre en post-levée des graminées) suivies par d'autres molécules ces dernières années, a restreint les options chimiques disponibles pour lutter contre les adventices (Agreste, 2023). Des dérogations temporaires sont néanmoins attribuées régulièrement pour les Antilles et La Réunion. La possible interdiction de l'usage du glyphosate ajoute une source d'inquiétude et un défi supplémentaire pour les planteurs.

Forte dépendance aux intrants importés

La Réunion, en raison de son caractère insulaire, est soumise à des contraintes géophysiques qui engendrent une forte dépendance vis-à-vis de l'extérieur et pèse sur son économie. L'éloignement des principaux centres économiques est l'une des principales raisons du coût élevé des matières premières et des intrants agricoles, ce qui se traduit par des coûts de production supérieurs à ceux des autres régions productrices (Le Fur et Youssouffa, 2023). La filière canne est ainsi particulièrement vulnérable face aux fluctuations des coûts des intrants agricoles. L'augmentation des prix des engrais ces dernières années, accentuée par la hausse des coûts du fret maritime, a mis en difficulté la filière poussant certains planteurs à réduire leurs investissements en fertilisation au détriment de la productivité de la culture (Agreste, 2023).

Nouvelles contraintes environnementales et sociétales

Face à l'apparition de nouvelles contraintes environnementales et sociétales, le secteur agricole doit s'adapter et diminuer l'impact des modes de production sur l'environnement tout en maintenant une agriculture économiquement viable. Parmi d'autres, la réduction des intrants chimiques (pesticides et engrais minéraux) est devenue une priorité. En France, ces demandes sociétales ont conduit à la création du plan Ecophyto à la suite du Grenelle de l'environnement en 2008. Revu à plusieurs reprises, le plan Ecophyto II+ est aujourd'hui en application et vise à réduire de 50% de l'utilisation des produits phytosanitaires d'ici 2025. Une telle diminution nécessite des changements profonds des systèmes canniers réunionnais, jusqu'à présent fortement dépendants des herbicides.

2.5. Développement de pratiques agricoles innovantes

Des projets de recherche ont vu le jour depuis une quinzaine d'années afin de concevoir et évaluer des pratiques de désherbage alternatif, comme les projets CanécoH (Canne-à-sucre Econome en Herbicides) ou AgriécoH portés par eRcane[1] (Mansuy et al., 2019). Dans ce cadre, divers systèmes sont testés en stations expérimentales ou en co-conception chez des agriculteurs : (i) optimisation de l'utilisation des herbicides, (ii) choix variétal, (iii) gestion de la paille (épaillage et fanage), (iv) désherbage mécanique, (v) désherbage thermique, (vi) insertion de plantes de services entre deux cycles de canne ou en association avec la canne.

Si la pratique traditionnelle de l'épaillage et du fanage de la paille se sont avérées efficaces pour maîtriser les adventices sans affecter le rendement de la canne, d'autres techniques nécessitent davantage de travaux avant d'être transférées et adoptées par les planteurs (Antoir et al., 2016). C'est le cas notamment des plantes de services en intercalaire de la canne qui, malgré une bonne efficacité dans le contrôle des adventices (Mansuy et al., 2019), peut régulièrement entraîner une baisse significative du rendement. Parmi ces plantes de service, un intérêt particulier est porté aux légumineuses envisagées comme une solution potentielle pour réduire l'utilisation d'intrants azotés en raison de leur capacité à fixer l'azote atmosphérique.

3. Les associations canne-à-sucre x légumineuses

3.1. Les légumineuses de services dans les systèmes canniers

Définition d'une plante de services

Le terme « plantes de services » regroupe des espèces végétales implantées avant ou pendant une culture principale, dans la parcelle ou à proximité et qui ont pour objectif d'améliorer les fonctions de l'agroécosystèmes (services écosystémiques). En plus de réguler la flore adventice, les plantes de service peuvent également réguler d'autres organismes nuisibles tels que les ravageurs et les maladies (Altieri et al., 2011; He et al., 2018; Kocira et al., 2020). De plus, elles peuvent avoir un impact positif sur le climat en stockant du carbone, et peuvent également améliorer la fertilité des sols, servir d'habitat pour les pollinisateurs et les auxiliaires de culture, et réduire les risques de pollution de l'eau (Koohafkan et al., 2012; Snapp et al., 2005). Elles n'ont toutefois pas une finalité productive, les services d'approvisionnement (alimentaire ou fourrages) n'étant pas recherchés en priorité.

Services attendus dans les systèmes canniers

A la Réunion, l'introduction d'une biodiversité choisie dans les systèmes de culture de la canne-à-sucre a d'abord été envisagée pour réduire l'utilisation des herbicides dans le cadre du plan EcoPhyto II+. L'utilisation de plantes de services en intercalaire de la canne-à-sucre a permis de limiter la croissance des adventices et de réduire l'indice de fréquence de traitement des

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[1] En collaboration avec le CIRAD, CerFrance, la Chambre d'Agriculture de la Réunion, les lycées agricoles de St Joseph et de St Paul et FDGDON.

herbicides de plus de 50% (Mansuy et al., 2019). Bien que limité à certains contextes, ce service est également recherché dans d'autres pays producteurs comme l'Inde (Bhullar et al., 2006; Kaur et al., 2015). Dans toutes les régions productrices, un intérêt croissant est également accordé aux légumineuses en tant que solution potentielle pour réduire l'utilisation d'intrants azotés, en raison de leur capacité à fixer l'azote atmosphérique. Les services attendus dans ce contexte sont une amélioration de la fertilité des sols, qu'elle soit chimique (Li et al., 2013), physique (Farhate et al., 2022) ou biologique (Malviya et al., 2021).

Mode d'insertion des légumineuses de service

Les légumineuses (et plus largement les plantes de service) peuvent être introduites à différentes périodes du cycle de culture de la canne. En période d'inter-culture (période de jachère de 2 à 4 mois entre deux plantations), les légumineuses permettent de rompre le cycle des adventices et de limiter les risques d'érosion du sol, exposé aux intempéries pendant cette période, tout en apportant de l'azote supplémentaire au système. Les effets positifs des légumineuses sur le contrôle des adventices et sur la nutrition de la canne lors de la plantation suivante ont déjà été largement étudié dans la littérature (Di Bella et al., 2021; Farhate et al., 2022; Otto et al., 2020; Tenelli et al., 2021; White Jr et al., 2020).

En association avec la canne (également appelé en intercalaire, Figure 5), les légumineuses sont utilisées pour recouvrir l'inter-rang avant la fermeture de la canne (vers 5-6 mois). La couverture permanente du sol permet d'étouffer les adventices par compétition pour les ressources (en particulier la lumière, Geetha et al., 2019). Cependant, le couvert semé peut être à l'origine de dis-service en entrant également en compétition avec la canne (Yang et al., 2013). Néanmoins, alors que les bénéfices des légumineuses en rotation ont été largement étudiée, les bénéfices en association sont encore peu étudiés en culture de canne-à-sucre.

Figure 5. Photos de légumineuses (*Canavalia ensiformis*, pois sabre blanc) semées en intercalaire de la canne-à-sucre à différents stades de croissance, en année de plantation (haut) ou de repousse (bas). Source : Cirad.

3.2. Les associations de culture : entre compétition, complémentarité et facilitation

Définition des interactions entre espèces

Dans les mélanges plurispécifiques, les interactions entre espèces se produisent via leur environnement selon différents processus, en particulier les processus de compétition, de facilitation et de complémentarité (Justes et al., 2021; Vandermeer, 1992). La compétition survient lorsque l'une des espèces modifie l'environnement de l'autre de manière à entraver son développement, pouvant être réciproque (compétition stricte) ou non (amensalisme). Cela se manifeste lorsque les plantes se disputent des ressources limitées telles que la lumière, l'eau, les nutriments ou l'espace racinaire. La compétition peut être réduite grâce à la complémentarité de niche, où une espèce accède à une ressource non disponible pour l'autre, comme dans les associations entre non-légumineuses et légumineuses, cette dernière pouvant capter l'azote atmosphérique. La facilitation améliore positivement les conditions environnementales pour la deuxième espèce, réduisant par exemple les maladies, les adventices (Teasdale, 1996) ou augmentant les ressources disponibles (par exemple l'azote et le phosphore) (Li et al., 2007; Oberson et al., 2013). La facilitation peut être réciproque (mutualisme) ou non (commensalisme).

Ces relations évoluent tout au long du cycle de l'association et même après (lors de la restitution de l'N des légumineuses au système sol-plante par exemple) et peuvent donc se manifester en même temps ou à des périodes distinctes durant l'association. Les interactions observées entre les plantes sont ainsi la somme des interactions positives et négatives entre les plantes (Callaway and Walker, 1997). Cet équilibre entre compétition et facilitation dépend de l'intensité des stress abiotiques, du stade de développement des espèces et de leurs physiologies (type de développement racinaire, port aérien, capacité à fixer l'azote atmosphérique) (Justes et al., 2014), mais également de leurs plasticités en réponse à la compétition (Ashton et al., 2010). Enfin, ces interactions peuvent être moduler par la gestion de l'itinéraire technique (choix des espèces associées, densité et l'arrangement spatial des espèces associées, date de semis et de destruction de la plante de service, irrigation et fertilisation).

Interactions entre les trois composantes du système canne x légumineuse x adventice

Dans les systèmes multi-espèces canniers, la compétition pour l'accès aux ressources joue un rôle crucial, ayant un impact direct sur la productivité globale du système. Par exemple, la nuisibilité des adventices en début de croissance de la canne-à-sucre a été bien documenté (Marion and Marnotte, 1991). Les grandes graminées comme les fataques (*Megathyrsus maximus*) aux caractéristiques proches de la canne sont connues pour être les adventices parmi les plus nuisibles (Martin et al., 2010). Afin de maîtriser efficacement les adventices, les légumineuses doivent montrer une forte compétitivité en couvrant adéquatement le sol et en développant une biomasse importante (Christina et al., 2021). Ce faisant, elles pourraient également entrer en compétition avec la canne-à-sucre, en particulier pour la lumière au début de la croissance de la canne. Néanmoins, du fait de traits différents, nous pourrions nous attendre à une forte complémentarité entre les légumineuses et la canne-à-sucre qui devrait limiter la compétition (source d'azote différentes, enracinement profond pour la canne et plus superficiel pour les légumineuses, Christina et al., 2023). Pour limiter cette compétition, il est également possible de décaler la date de semis des légumineuses par rapport à la période de récolte de la canne en donnant un avantage compétitif à cette dernière (Mansuy et al., 2019). Cependant, une compétition excessive de la canne envers les légumineuses pourrait alors nuire à leur croissance et réduire les services attendus. Ainsi, la gestion adéquate de ces interactions entre cannes, légumineuses et adventices est essentielle pour optimiser les performances du système de culture.

3.3. Les associations canne-à-sucre x légumineuses : des connaissances fragmentées

En comparaison d'autres grandes cultures, les interactions interspécifiques au sein des associations canne x légumineuses ont encore été peu étudiées. Dans la littérature, l'impact des légumineuses sur les rendements de canne varient très fortement d'une étude à l'autre, autant dans le sens d'une augmentation des rendements que d'une baisse, en comparaison d'une culture pure (Ambrosano et al., 2013; de Resende et al., 2003; Nadeem et al., 2020; Tian et al., 2020). De manière similaire à d'autres grandes graminées, cette réponse pourrait être influencée par le climat, le sol ou l'itinéraire technique (Waddington et al., 2007; Xu et al., 2020). Néanmoins l'influence de ces facteurs sur les rendements canniers et les processus de compétition qui sont en jeux sont encore mal compris. De plus, à l'exception de l'utilisation de la légumineuse comme culture de rente supplémentaire (e.g. Nadeem et al., 2020), les services apportés par la légumineuse en association avec la canne sont encore peu étudiés, en particulier pour ce qui est des conséquences sur la fertilité azotée du système (Lian et al., 2019; Wang et al., 2020).

4. Objectifs et démarche de la thèse

4.1. Objectifs et hypothèses de recherche

Cette thèse s'inscrit dans un objectif général d'enrichir les connaissances sur les interactions de compétition et/ou facilitation dans le système multi-espèces canne-à-sucre x plantes de services x adventices. Une meilleure compréhension du fonctionnement complexe de ces associations permettra de mieux définir les avantages potentiels de l'introduction de légumineuses dans la culture de la canne-à-sucre et de développer des stratégies de gestion efficaces. Ce travail de thèse se focalise sur l'étude des interactions entre canne-à-sucre et légumineuse. En parallèle, d'autres travaux de recherche auquel j'ai participé s'intéressent aux interactions entre canne-à-sucre et adventices (Thèse en cours de Léa Chevalier ; Christina et al., 2023), ainsi que celles entre plantes de service et adventices en couvert ((Christina et al., 2021; Négrier et al., 2023; Soulé et al., under review). Les publications issues de ces travaux sont incluses en annexe de ce manuscrit.

L'objectif principal de la thèse est de caractériser les interactions de compétition et de facilitation entre canne-à-sucre et légumineuse.

Plus spécifiquement, le premier objectif de la thèse est de **caractériser l'impact des légumineuses sur la croissance et le rendement de la canne** en répondant aux questions suivantes : Les légumineuses sont-t-elles plus nocives que les adventices pour la canne-à-sucre ? Les légumineuses exercent-t-elles une pression compétitive sur la canne-à-sucre, ou à l'inverse supportent-elles sa domination durant le cycle de production ? S'il y a une compétition, quelle est la réponse de la canne à la compétition et comment cela affecte t'il l'élaboration du rendement ? Enfin, comment la disponibilité des ressources (lumière, eau et azote), lié au contexte pédoclimatique et à l'itinéraire technique, influence ces interactions ?

- **Hypothèse 1** : La compétition d'une plante choisie est moins forte que celle d'une communauté d'adventices ayant des stratégies d'acquisition des ressources différentes et complémentaires.
- **Hypothèse 2** : L'insertion d'une légumineuse en intercalaire de la canne-à-sucre modifie la disponibilité des ressources du milieu, ce qui peut affecter la croissance de la canne-à-sucre (et des adventices), et in fine son rendement.
- **Hypothèse 3** : Les interactions entre les espèces dépendent des dynamiques de croissance aérienne et racinaire des espèces, influencés par la disponibilité des ressources.
- **Hypothèse 4** : La direction et l'intensité de la compétition peuvent être influencées par certaines techniques culturales : irrigation, fertilisation, date de semis et de destruction du couvert.

Le second objectif de la thèse est d'**évaluer l'intérêt des légumineuses pour la nutrition de la canne et la fertilité du sol** en répondant aux questions suivantes : Quelle part de l'azote accumulée par les légumineuses durant leur croissance provient de l'atmosphère ? Existe-t-il une facilitation pour la ressource azote pour la canne-à-sucre au cours du cycle de culture et/ou lors des cycles suivants ?

- **Hypothèse 5** : La quantité d'N^2 fixé par la légumineuse dépend de sa capacité à croître. La proportion d'N^2 fixé peut être modifié par la gestion de l'itinéraire technique (fertilisation azotée et irrigation).
- **Hypothèse 6** : La décomposition rapide des résidus de légumineuse en cours de culture permet d'augmenter l'offre en azote pour la canne-à-sucre au cours du même cycle.
- **Hypothèse 7** : Une partie de l'azote issus de la décomposition de la légumineuse est intégré à la matière organique du sol et est restitué à la canne-à-sucre au cours des cycles suivants.

4.2. Démarche adoptée

Impact des légumineuses sur le rendement en canne en fonction des conditions agro-pédoclimatiques.

Le premier volet de la thèse a été dédié à la réalisation d'une **méta-analyse dans le but de déterminer et quantifier l'impact des légumineuses sur le rendement de la canne-à-sucre, en fonction du contexte pédoclimatique et de l'itinéraire technique**. Cette étude a permis de synthétiser et de valoriser les données recueillies ces dernières années à La Réunion, grâce aux travaux de recherche menés par eRcane et le CIRAD. Ces données proviennent d'un réseau de huit d'essais mené sur les associations canne-à-sucre x plantes de services, dans lequel différentes espèces de légumineuses, dates de semis et date de destruction du couvert, ont été testées. Pour élargir la portée de l'étude, cette base de données a été complété par une revue systématique de la littérature, permettant ainsi d'inclure une gamme plus large de conditions agro-pédoclimatiques. Cette étude correspond au premier chapitre du manuscrit, publié dans Field Crop Research (Viaud et al., 2023).

Répartition des ressources (lumière, eau, azote) entre canne et légumineuse dans un cas d'étude.

Le second volet s'intéresse plus finement aux processus de répartition des ressources (azote, eau, lumière) entre la canne et la légumineuse dans un agrosystème de référence. L'objectif est de **comprendre comment la canne répond à la présence des légumineuses à différentes étapes de son cycle de croissance et selon différents niveaux de stress (eau et azote) et comment cela se répercute sur le rendement**. Ce volet repose sur le suivi d'un essai instrumenté consistant en un essai factoriel croisant les traitements : *Canavalia ensiformis* en intercalaire de la canne ou monoculture de canne, fertilisation en azote ou non, irrigation ou non. L'essai a été planté en 2018 sur la station expérimentale de la Mare au Nord de l'île. Il a été suivi pendant 3 cycles de culture (Repousses 1 à 3). L'itinéraire technique a été choisi à partir des essais précédant menés à La Réunion (date de semis, densité et arrangement spatial, (Mansuy et al., 2019). Le choix de la légumineuse (*Canavalia ensiformis*, pois sabre blanc) est fondé sur un réseau d'essais de collection de plantes de service en couvert (Christina et al., 2021) ou en intercalaire de la canne ((Mansuy et al., 2019; Soulé et al., under review) mené à La Réunion par eRcane et le CIRAD depuis une dizaine d'année.

Intérêt des légumineuses pour la nutrition de la canne et la fertilité du sol

Le dernier volet s'intéresse au **devenir de l'azote issu de la fixation dans le système canne x légumineuse x sol**. Ce volet repose sur le suivi du même cas de référence défini précédemment (canne x pois sabre blanc) pendant trois ans. Le taux de fixation de l'azote par la légumineuse, sa vitesse de décomposition en interaction avec le mulch de canne et enfin le devenir de cet azote en termes de prélèvement par la canne, immobilisation dans le sol ou différentes pertes ont été évalués. Cette évaluation a principalement reposé sur l'analyse isotopique de l'azote (^{15}N) à travers des mesures d'abondance naturelle (pour la fixation) ou d'enrichissement pour le devenir de l'azote dans le système.

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Chapter 1: Sugarcane yield response to legume intercropped: a meta-analysis.

Ce travail a été réalisé au sein des unités de recherche AIDA et Recyclage et Risque du Cirad de Saint-Denis de La Réunion. Ce chapitre correspond à un article publié dans Field Crop Research (Viaud et al., 2023). Les personnes impliquées ainsi que leurs contributions respectives à ce chapitre de thèse sont listées ci-dessous.

Pauline Viaud a conceptualisé la méthodologie analytique, collecté et analysé les données, et effectué la rédaction du chapitre. **Benjamin Heuclin** et **Philippe Letourmy** ont apporter leurs compétences pour définir la méthodologie analytique, et ont participé à l'analyse statistique des données, à l'interprétation des résultats et à la rédaction du chapitre. **Mathias Christina**, **Krishna Naudin** et **Antoine Versini** ont participé à la conceptualisation de la méthodologie analytique, à l'interprétation des résultats et à la rédaction du chapitre. **Alizé Mansuy** et **Julien Chetty** (eRcane) ont mis à disposition leurs jeux de données et participer à l'interprétation des résultats.

Abstract

Intercropping with legumes has attracted much attention worldwide, regarded as a sustainable alternative to chemical N-fertilizer. Many studies have investigated the effects of introducing legumes into sugarcane systems. However, how agro-environmental conditions influence the effect of legumes on sugarcane yield is still unclear. Thus, we performed a meta-analysis to i) quantify the effect of legume intercropping on sugarcane yield, ii) assess how agro-environmental and management conditions influence the response of sugarcane yield to intercropping, and iii) assess the influence of legumes in interaction with weeds on sugarcane yield. We combined data from a systematic literature review and experimental data from a network of experiments conducted in the tropical island of La Réunion. 315 observations (one-year x site x intercropping treatment) were analyzed with mixed effects models to assess the effect of legume intercropping on sugarcane yield and factors influencing the response of sugarcane yield. Intercropping with legumes resulted in a 3.34% lower sugarcane yield on average compared to the monoculture treatment, but with high variation from -65 to +47%. Our study highlighted how the competition of legumes on sugarcane yield is reduced by higher annual temperatures and soil organic carbon. Late sowing and early destruction date of the legume mitigated the negative effect of legumes on sugarcane yield. Our study also showed that the negative impact of legumes on sugarcane yield increased over years of intercropping. Further studies are needed to better synchronize legume sowing and destruction dates while reducing trade-offs between expected services (N enrichment and weed control) and disservices (yield loss, costs, and labor time) in sugarcane agrosystems according to the climate and soil context.

Highlights:

- Legume intercropping reduced sugarcane yield by an average of 3.34% worldwide.
- Resource availability influenced sugarcane yield response to legumes.
- Legumes sowing and destruction dates drove the competitiveness between species.
- Legumes were twice less damaging than weeds for sugarcane.
- The negative effect on yield increased with the number of years of intercropping

1. Introduction

Under the extensive use of chemical inputs in the 20th century, the intensification of agriculture has led to the emergence of environmental issues such as soil degradation, water pollution, eutrophication, greenhouse gas emissions, biodiversity loss, or weeds' resistance to herbicides (Bengtsson et al., 2005; Heap, 2014; Howarth, 2008; Jie et al., 2002). The environmental cost of intensification raises the question of the sustainability of this agricultural model (Sutton et al., 2013).

Sugarcane is a worldwide crop cultivated for sugar, biofuel, and material for electric energy production (Antunes et al., 2019). The demand for ethanol and electricity will likely increase in the energy sector as it could replace fossil fuels and reduce greenhouse gas emissions (Goldemberg et al., 2014; Leal et al., 2013). Sugarcane is a semi-perennial crop that relies heavily on nitrogen (N) fertilization (FAO 2006). However, the amount of N fertilizer taken up by the sugarcane is particularly low, ranging from 10% to 40% of the total amount applied (Otto et al., 2016). As a result, concerns about the low N-use efficiency and environmental impacts of N losses in these systems have been raised (Thorburn et al., 2017; Wang et al., 2020). Therefore, there is a need to develop sustainable agricultural management to maintain economically viable production levels of sugarcane with less reliance on chemical N-fertilizer input.

Legumes are a potential alternative to synthetic fertilizers thanks to their ability to fix atmospheric N (Ranaivoson et al., 2022). Legumes can be used as (i) cover crops, i.e., plants grown in the field between two crop cycles, or (ii) as companion plants in intercropping systems, i.e., plants grown with a cash crop in the same space for a significant part of their growing period (Verret et al., 2017). Many studies have considered the positive effect of legumes as a rotation break crop in sugarcane systems to minimize the requirements for N fertilization by the following plantations (Garside & Bell, 1999; Hemwong et al., 2009; Shoko et al., 2007). They showed that legumes could limit mineral N inputs without decreasing sugarcane yield at planting. However, sugarcane is commonly planted every 7 to 15 years, limiting legumes' positive effects as a rotation break crop.

In recent years, many studies have been carried out to investigate the effect of companion plants intercropped, such as legumes, in sugarcane systems. Intercropping may reduce the combined area required for food production while providing additional ecosystem services leading to more sustainable land use (e.g., Della Rossa et al., 2022; Pelzer et al., 2012). In particular, intercropping with legumes may reduce N fertilization and N_2O emissions (Jensen et al., 2020). Additionally, legumes may compete with weeds and help reduce herbicide use (Verret et al., 2017). However, excessive interspecific competition can lead to a significant yield loss of the main cash crop (Justes et al., 2021). As observed in other annual intercropping systems (e.g., in maize: Sileshi et al., 2008; in maize, sorghum, and pearl millet: Namatsheve et al., 2020), the impact of legumes on sugarcane yield varies significantly from one study to another (Ambrosano et al., 2013; de Resende et al., 2003; Nadeem et al., 2020; Tian et al., 2020). In perennial crops, legume intercrops with high biomass can reduce cash crop performance (e.g., banana, Ocimati et al., 2019) but also sometimes improve the quality of harvest (e.g., vine, Mercenaro et al., 2014). The impact of legume intercropping on sugarcane yield could vary depending on climate (Waddington et al., 2007), soil

(Xu et al., 2020), management practices (e.g., crop species and growth habit, fertilization, and irrigation regime, crop density, the time and duration of cohabitation, Pelzer et al., 2014; Yu et al., 2016, 2015). The interactions in species mixtures are complex, with key issues regarding competition and the timing of N restitution to the main crop (Justes et al., 2014). Apart from sugarcane and companion plants, a third component, the weed community, increases the complexity of these multi-species interactions.

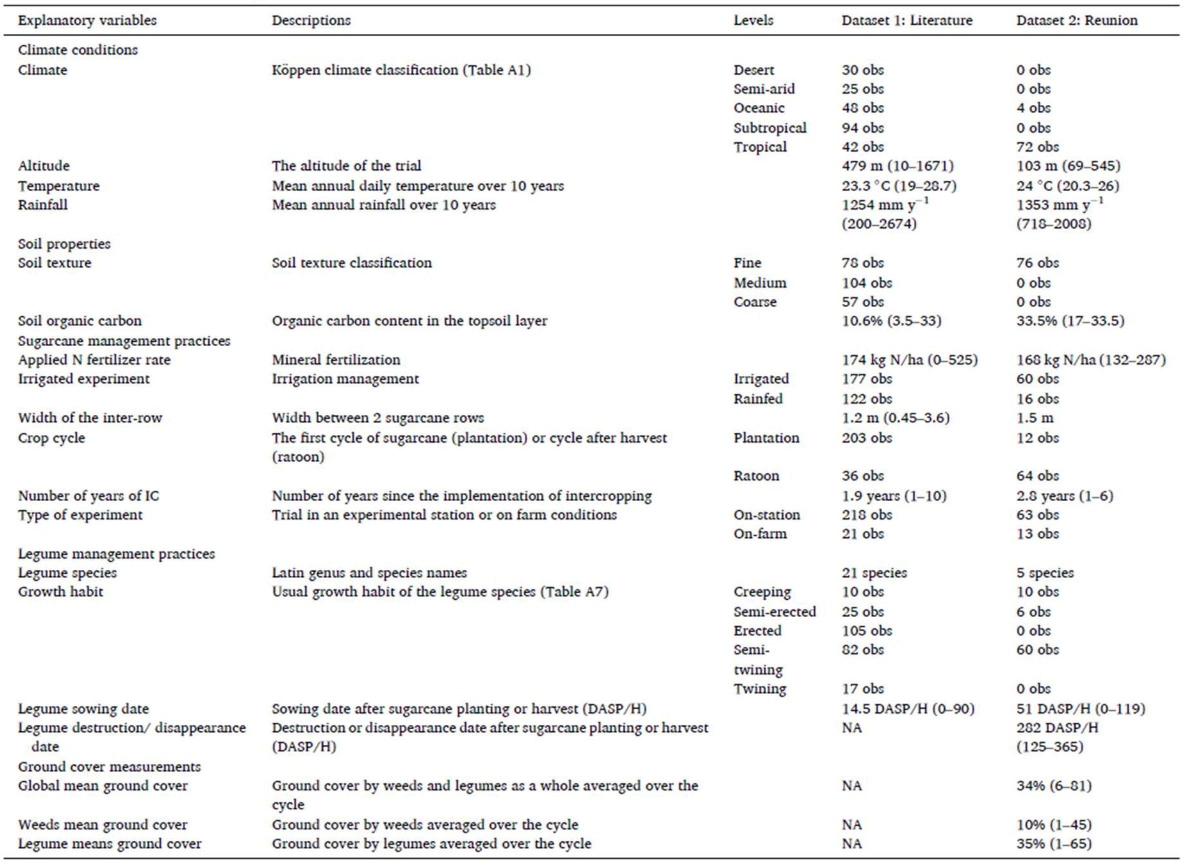
The diversity of the results observed in sugarcane yield response to legume grown as a companion plant in the inter-row highlights the need for a comprehensive and quantitative analysis. To our knowledge, the impact of climate, soil, and management practices variability on sugarcane yield response to legume intercropping has never been analyzed in a quantitative study. The objectives of this study were i) to quantify the effect of legume intercropping on sugarcane yield, ii) to assess how agro-environmental and management conditions influence the response of sugarcane yield to intercropping, and iii) to assess the influence of legumes in interaction with weeds on sugarcane yield. We performed a meta-analysis by combining data from a literature review and experimental data from a network of experiments conducted in the tropical island of La Réunion.

2. Materials and methods

2.1. Complementarity of the two data sets

A meta-analysis was performed to assess the sugarcane yield response to row legume intercropping compared to sugarcane grown as a monocrop. The database included two complementary data sets. The first data set was based on a systematic literature review (section 2.2, Table 1). This data set had the advantage of covering a wider range of agro-pedo-climatic conditions but the disadvantage of having less information on crop and legume management. The second data set included data from a network of experiments performed in the Island of La Réunion (section 2.3, Table 1). This data set had the advantage of providing more information than the first one (e.g., actual temperature and rainfall, destruction/disappearance day of the companion plant, and ground cover by legumes and weeds).

Table 1. Variables used in the meta-analysis from the two data sets. The number of observation (obs) or mean (minimum and maximum) values in the datasets are presented. The characteristics of the climate types are shown in Table A1. NA: Not available for the data set.



Explanatory variables	Descriptions	Levels	Dataset 1: Literature	Dataset 2: Reunion
Climate conditions				
Climate	Köppen climate classification (Table A1)	Desert	30 obs	0 obs
		Semi-arid	25 obs	0 obs
		Oceanic	48 obs	4 obs
		Subtropical	94 obs	0 obs
		Tropical	42 obs	72 obs
Altitude	The altitude of the trial		479 m (10–1671)	103 m (69–545)
Temperature	Mean annual daily temperature over 10 years		23.3 °C (19–28.7)	24 °C (20.3–26)
Rainfall	Mean annual rainfall over 10 years		1254 mm y^{-1} (200–2674)	1353 mm y^{-1} (718–2008)
Soil properties				
Soil texture	Soil texture classification	Fine	78 obs	76 obs
		Medium	104 obs	0 obs
		Coarse	57 obs	0 obs
Soil organic carbon	Organic carbon content in the topsoil layer		10.6% (3.5–33)	33.5% (17–33.5)
Sugarcane management practices				
Applied N fertilizer rate	Mineral fertilization		174 kg N/ha (0–525)	168 kg N/ha (132–287)
Irrigated experiment	Irrigation management	Irrigated	177 obs	60 obs
		Rainfed	122 obs	16 obs
Width of the inter-row	Width between 2 sugarcane rows		1.2 m (0.45–3.6)	1.5 m
Crop cycle	The first cycle of sugarcane (plantation) or cycle after harvest (ratoon)	Plantation	203 obs	12 obs
		Ratoon	36 obs	64 obs
Number of years of IC	Number of years since the implementation of intercropping		1.9 years (1–10)	2.8 years (1–6)
Type of experiment	Trial in an experimental station or on farm conditions	On-station	218 obs	63 obs
		On-farm	21 obs	13 obs
Legume management practices				
Legume species	Latin genus and species names		21 species	5 species
Growth habit	Usual growth habit of the legume species (Table A7)	Creeping	10 obs	10 obs
		Semi-erected	25 obs	6 obs
		Erected	105 obs	0 obs
		Semi-twining	82 obs	60 obs
		Twining	17 obs	0 obs
Legume sowing date	Sowing date after sugarcane planting or harvest (DASP/H)		14.5 DASP/H (0–90)	51 DASP/H (0–119)
Legume destruction/ disappearance date	Destruction or disappearance date after sugarcane planting or harvest (DASP/H)		NA	282 DASP/H (125–365)
Ground cover measurements				
Global mean ground cover	Ground cover by weeds and legumes as a whole averaged over the cycle		NA	34% (6–81)
Weeds mean ground cover	Ground cover by weeds averaged over the cycle		NA	10% (1–45)
Legume means ground cover	Ground cover by legumes averaged over the cycle		NA	35% (1–65)

2.2. Literature review

A literature search was performed using the online database Scopus (Elsevier) in June 2021. The search equation was (an asterisk being a replacement for any starting/ending of the respective term) : (legum* OR faba* OR pulse* OR "cover crop" OR "green manure*" OR Arachis OR Cajanus OR Canavalia OR cicer OR crotalaria OR glycine OR lens OR phaseolus OR Pisum OR vicia OR vigna) AND (*cane OR "Saccharum officinarum") AND (intercrop* OR "crop association" OR "associated crops" OR "companion crop*" OR "mixed crop*" OR mixtures OR "multiple crops*") in the title, abstract or keywords. The database searches were increased with searches in library resources for relevant papers from citations in retrieved papers.

Studies had to meet the following selection criteria to be included in the meta-analysis: (i) studies report sugarcane yield from a field experiment in which a monoculture treatment (CT) was compared with at least one legume-intercropped treatment (IC), (ii) the mean and number of replicates were directly reported as numerical or graphical data, (iii) full-text articles were available. Data reported as an average across years, locations, or factors were excluded. We did not include treatments with more than one legume species (e.g., legume mixture or two species planted one after the other in the same crop cycle). Studies were excluded if crop management practices other than intercrop practices differed between CT and IC, except for weed management. If data from the same experiment were reported in several papers, the data were included from the article that reported the data in the most significant degree of detail. The literature search and the selection procedure were presented in a Prisma diagram (Appendix A, Fig. A1).

A total of 31 papers fit our selection criteria for the meta-analysis (Table A2). The resulting database included 39 experiments (one site x multiple years x multiple IC treatments) and 239 observations (one-year x site x IC treatment) from the literature review.

2.3. Network experiments in La Réunion

A network of eight experiments was carried out to assess the ability of legume companion plants intercropped with sugarcane to control weeds in the inter-row and their impact on sugarcane yield from 2012 to 2019. Depending on experiments, different legume species, legume sowing dates, and destruction dates have been tested (Table A2 to A6).

Recommended non-limiting fertilizer rates were applied to sugarcane for all experiments (Table A5). All experiments were irrigated except those in Saint-Benoît with enough annual rainfall. Details on experimental design and crop management are available in Table A3 to A6. Ground cover by legume-was measured using a visual notation method used in previous studies (Christina et al., 2021a) described in Table A7. Global ground cover by legume species and weeds, considered as a whole, was measured monthly in the inter-row. Respective ground covers by legume species and weeds were calculated by multiplying the global ground cover by the respective proportion of each pool. The measure was extrapolated between two measurement dates to estimate a mean ground cover over the sugarcane crop cycle. Sugarcane stalk fresh mass (hereafter sugarcane yield) was measured at harvest by sampling all sugarcane stalks on the two rows in the middle of the plot (i.e., a minimum of 30 m^2 areas). The resulting database contained 76 observations (one-year x site x treatment) from the Reunion network experiments.

2.4. Database

We compiled and harmonized the data from the literature and the Reunion network experiments in a database. We collected the means sugarcane yield of the control treatment (CT) and the intercropped treatment (IC), their standard deviation, and replicate numbers. Standard deviations were calculated from standard errors, coefficients of variation, 95% confidence interval, or least significant differences in cases that were not reported in the papers.

To explain the variability in sugarcane yield responses due to legume intercropping, we considered several explanatory variables related to climate, soil, and management practices. The explanatory variables used in the meta-analysis are shown in Table 1. Information for these variables was extracted from the text, tables, or figures using a web-based image analysis, Webplotdigitizer v4.5 (Rohatgi, 2018). When soil organic matter was reported, we converted it to soil organic carbon by dividing the value by 2 (Pribyl, 2010). If not reported, average annual rainfall and annual temperature data over 10 years were retrieved using the New_LocClim 1.10 database (Grieser, Gommes, and Bernardi 2006). The legume species were classified according to their usual growth habit (creeping, erected, semi-erected, semi-twining, and twining) as identified by the Useful Tropical Plants (https://tropical.theferns.info/) and the PROTA4U (https://www.prota4u.org/) databases (Table A8).

Some variables had a few missing data (< 15%). We used single imputation method to include them in the analysis (Scheffer 2002). The country's median was imputed when the applied N fertilizer rate for sugarcane was missing (31/315 observations). Part of the soil carbon content of the topsoil layer (%) was missing (46/315) for coarse soils. As the soil C content was significantly influenced by soil texture in our dataset (as in Conforti et al., 2016), the mean value of C content for coarse soils was imputed when missing. Some of the variables initially considered were omitted for the analysis of the literature data because there were too many missing values (actual rainfall and temperature during the experiment, applied NPK fertilizer for legumes, applied PK fertilizer for sugarcane, use of herbicide with IC, legume' destruction/disappearance day of legume).

2.5. Data analysis

2.5.1. Linear mixed model

Three mixed-effect models were built to estimate the effect of legumes and explanatory variables on sugarcane yield for each subset of the database: Model 1: Full data set (Literature + Reunion); Model 2: Literature data set; Model 3: Reunion data set. As the observations from La Réunion were over-represented in the database, comparing models 1 and 2 allowed us to verify that these observations did not induce a bias in our analysis. The third model was built to consider the variables not or not widely available in the literature. The approach used to build these models is described in the following sections.

2.5.2. Effect size and weight of the observations

The natural logarithm of the ratio of IC-to-CT sugarcane yields was used as the effect size in the meta-analysis (Hedges, Gurevitch, and Curtis 1999):

$$L = \log\left(\frac{Yield_{IC}}{Yield_{CT}}\right)$$

Where Yield_{IC} and Yield_{CT} are the sugarcane yield in the intercropping and control treatment, respectively. The results were back-transformed and presented as the percent change in sugarcane yield of the IC treatment compared to the CT treatment in the figures and results. The number of replicates was used to weight observations to ensure robustness and statistical significance by keeping a sufficient sample size. In meta-analysis, observations were commonly weighted by the inverse of their variance. Unfortunately, this information was missing in many selected studies (86 obs from 8 papers). In addition, some of the selected studies had a very low coefficient of variation (< 5%) compared to those usually reported for sugarcane yields (Fig. A2). As a comparison, the average coefficient of variation of the Reunion network experiments was 11.91 ± 1.05 %.

2.5.3. Sensitivity and publication bias analysis

A first exploration showed that the full data set and the literature had a non-Gaussian residual distribution (Fig. A3). To correct it, observations with a Cook distance greater than 4/n were eliminated from the data set (with n=number of observations) (Viechtbauer and Cheung 2010). A new literature data set was extracted from it. Alone, the Reunion data set did not require a selection based on Cook distance. Finally, the full, literature, and Reunion data sets contained respectively 281, 212, and 76 observations (Fig. A3). The observations from He et al. (2018) had a unique behavior compared to other studies regarding nitrogen fertilization effect on the response variable (Fig. A4). An indicator variable, "He2018" was created to take it into account. Publication bias was assessed by drawing funnel plots, in which standard errors were plotted against the effect size log-ratio (Fig. A8, Duval and Tweedie 2000). Publication bias was tested on data with a reliable coefficient of variation (>5%). Funnel plot asymmetry was tested with a rank correlation test (Begg and Mazumdar 1994).

2.5.4. Data analysis

The "experiment" (ensemble of individual IC observations from the same experiment – one site x multiple years) and "same_control" factors (ensemble of individual IC observations with the same control treatment in a given site and year) were tested as random effects to account for the hierarchical correlation between multiple observations within a study (Lajeunesse 2011). The best random structure was identified by comparing Akaike's information criterion (AIC) of models with different random structures and the same fixed structure with all variables (model beyond the optimal) using the restricted maximum likelihood (REML) estimation (Zuur et al., 2009). A first-order autocorrelation structure AR(1) was also tested to account for repeated observations not being independent over the years within one experiment. Finally, the best random structure including only the "same_control" effect.

The best fixed effect structure was identified by compared the Akaike's information corrected criteria (AICc) of models with all possible combinations of explanatory variables set included interaction. Models were fitted using maximum likelihood (ML) estimation (Zuur et al., 2009). The best-fitted models were presented in Table 2, as follows:

$$L_{ij} = \mu + (\sum_{m} a_m * {X_{ij}}^{m}) + b_i + \varepsilon_{ij}$$

Where L_{ij} is the effect size for observation *j* from the "same_control" *i*, μ is the intercept, X_{ij}^m is the *m* explanatory variables with their coefficients a_m, b_i is the random effect ("same_control"), and ε_{ij} the residual. In model 1, X_{ij}^m included one interaction between climate type and mean annual temperature.

Finally, the parameters of the resulting model were fitted using REML estimation. The assumptions of homogeneity and normality were checked graphically using histograms of residuals, quantile-quantile plots of Pearson's residuals versus standardized Gaussian sample, and plots of residuals against fitted values, respectively (Fig. A5 to A7).

All analyses were performed in R 4.1.1 (R Core Team, 2021) except the test of the autocorrelation structure performed in SAS 9.3 (SAS Institute, Cary, NC) using the PROC MIXED procedure. In R, linear mixed models were performed with the function lme() from the package nlme (Pinheiro et al., 2021). For categorical variables, pairwise comparisons were performed with the function lsmeans() from the package emmeans (Lenth et al., 2018). The rank correlation tests were performed with the function ranktest() from the package metafor (Viechtbauer 2010).

The data used in this study are available from the Dataverse repository in Viaud et al. (2022).

3. Results

3.1. Sugarcane yield response to intercrop treatment

The effect of legumes intercrops on sugarcane yields varied widely, ranging from a 65% increase to a 47% decrease compared to the monoculture treatment (Fig. 1). In the full data set, the mean IC-to-CT yield ratio was -3.3 %. In 22% of observations, legumes had a strong depressive effect on sugarcane yield (IC-to-CT yield ratio < -10%). In 13% of observations, legumes had a strong positive effect on sugarcane yield (IC-to-CT yield ratio > 10%). The mean IC-to-CT yield ratio was lower in the Reunion data set (-5.8%) than in the literature data set (-2.5%). Several outliers were found in the full data set with Cook's distances. When outliers were removed, the mean IC-to-CT yield ratios were -2.2, -1.0, and -5.8 %, respectively, for the full, literature, and Reunion data set (Appendix B, Fig. B1). The symmetrical funnel plot suggested no publication bias in our study (Fig. A8). However, this result must be considered cautiously because we could draw the funnel plot only on a restricted part of the data (99/315), excluding data for which the variance was unknown and data with a coefficient of variation <5%.

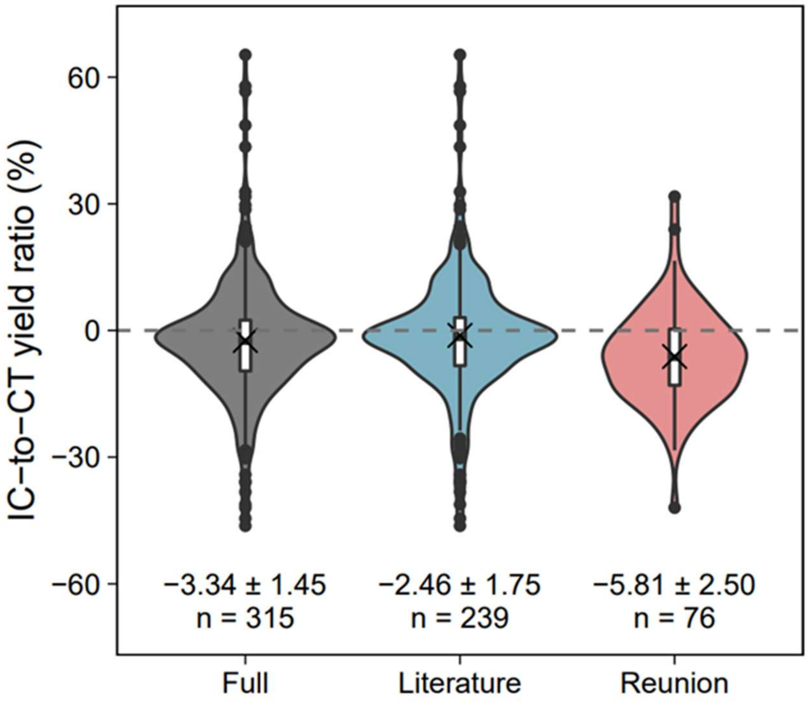


Fig. 1. The IC-to-CT yield ratio depending on the data sets. The violin plots represent the data distribution. The horizontal lines within the boxes indicate medians, and the crosses indicated the mean. The upper and lower edges of the boxes indicate the 75th and 25th percentiles, and the whiskers below and above the boxes indicate the 10th and 90th percentiles. Outliers are plotted as individual points. The mean value, confidence intervals (±), and the number of observations (n) are indicated below the violin plots.

3.2. Explanatory variables selected for the three models

All three models explained a significant proportion of the IC-to-CT yield ratio variability with conditional coefficient of determination values (R^2) of 0.74, 0.77, and 0.77 in models 1, 2, and 3, respectively (Table B1 to B3). Selected explanatory variables differed between models (Table 2).

Table 2. Analysis of variance (ANOVA) of the random-effects models built to assess the influence of explanatory variables on the IC-to-CT yield ratio for the three data sets. The p values and F values are indicated. NA = not available for the data set, boxes left blank = variables not selected based on the AICc criteria.

Variables	Full Model 1	Literature Model 2	Reunion Model 3
Climate	F = 6.24, P = 0.0002	F = 3.95, P = 0.0057	
Mean annual temperature	F = 3.45, P = 0.0650	F = 18.12, P = 0.0001	
Climate x Temperature	F = 6.59, P = 0.0001		
Mean annual rainfall			
Altitude	F = 3.5, P = 0.0633		
Soil texture		F = 2.24, P = 0.0762	NA
Soil organic carbon		F = 2.66, P = 0.1383	F = 8.88, P = 0.0074
Applied N fertilizer rate			
Irrigated trial			
Legume species			
Growth habit	F = 2.13, P = 0.0798		
Planding day of CC	F = 3.26, P = 0.0728	F = 2.60, P = 0.1093	
Destruction day of CC	NA	NA	F = 7.87, P = 0.0071
Width of the interrow	F = 3.11, P = 0.0799		NA
Crop cycle		F = 3.08, P = 0.0831	
Number of years of IC	F = 3.08, P = 0.0823	F = 2.27, P = 0.1364	
Type of trial			
Global mean ground cover	NA	NA	
Weeds mean ground cover	NA	NA	F = 7.19, P = 0.0098
Legume mean ground cover	NA	NA	F = 5.71, P = 0.0206

3.3. Climate and soil effects

The interaction of temperature and climate significatively influenced the IC-to-CT yield ratio in the full data set (Model 1, Table 2). In Figures 2 to 5, the IC-to-CT yield ratio was computed with predicted values from models 1 to 3. The IC-to-CT yield ratio increased with the temperature in all climates except for tropical climate (Fig. 2a). The yield ratio response in the desert (5 studies), semi-arid (4 studies), and oceanic climates (2 studies) should be interpreted carefully due to limited observations (Fig. 2a). Increased temperature (up to 24°C) in subtropical climate may increase the effect on the IC-to-CT yield ratio. Above 24°C, in the tropical climate, the yield ratio did not change with temperatures. While the interaction between temperature and climate was not selected in the literature data set (Model 2, Table 2), the temperature and the climate significantly influenced the IC-to-CT yield ratio. The effects of these variables were similar to the results obtained in the full data set (Fig. B2).

The IC-to-CT yield ratio significantly decreased with the altitude in the full data set (Model 1, Table 2, Fig. 2b). On the contrary, no response to yield ratio to rainfall was found. However, the IC-to-CT yield ratio tended to increase with the mean annual rainfall for the rainfed experiments while decreasing in the irrigated ones (Fig. B3). The IC-to-CT yield ratio was also significantly influenced by the initial amount of C in the topsoil layer in both the literature (Model 2 Table 2) and the Reunion data sets (Model 3, Table 2). In both cases, the IC-to-CT yield ratio increased with the soil organic carbon (Fig. 2c). This increase was higher in the Reunion data set than in the Literature data set.

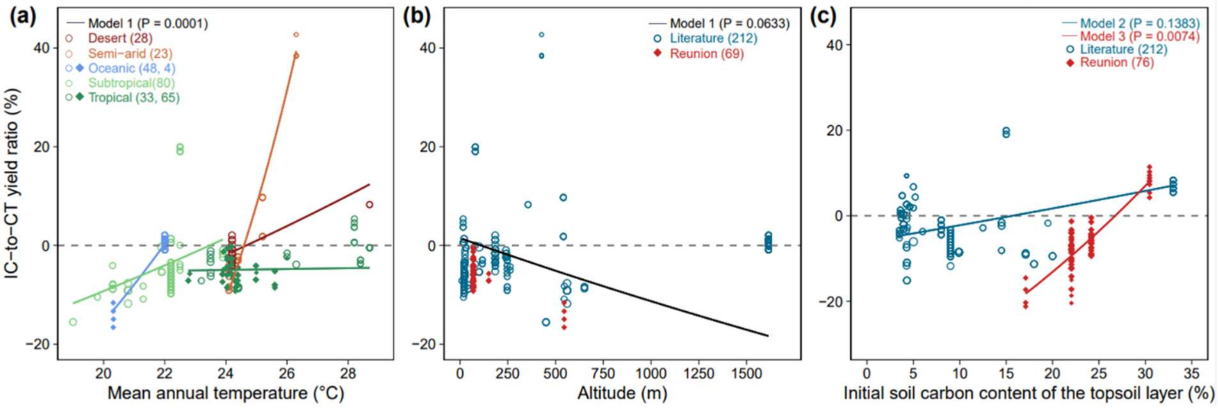


Fig. 2. The IC-to-CT yield ratio depending on (a) the mean annual temperature (°C) in interaction with the climate, (b) the altitude (m), and (c) the initial soil carbon content of the topsoil layer (%). Lines in (a) represent the fitted random effects model regression in model 1. Black, blue, and red lines in (b) and (c) represent the fitted random-effects model regression in models 1, 2, and 3, respectively. The open circles and filled points represent the literature and Reunion predicted values, respectively. The p.value and the number of observations are indicated in parentheses.

3.4. Management effects

Depending on the data sets, the IC-to-CT ratio was significantly influenced by the legume sowing date, the destruction or disappearance date of the legume, and the width of the sugarcane inter-row (Table 2). The sowing date of the legume was a significant covariate in the full data set (Model 1) and literature data set (Model 2) but was not selected in the Reunion data set (Table 2). In both cases, our results showed a strong negative effect of legumes on sugarcane yield for early sowing (Fig. 3a, B4). The destruction/disappearance date of the legume was a significant covariate in the Reunion data set (Model 3, Table 2) but was not available in the literature. The IC-to-CT yield ratio decreased with the destruction date (Fig. 3b). Finally, the IC-to-CT yield ratio decreased with an increase in the inter-row width in the full data set (Model 1, Table 2, Fig. 3c).

The legume species growth habit and the type of sugarcane cycle (plantation vs. ratoon crop) were selected for the full data set (Model 1) and the literature data set (Model 2, Table 2), respectively. However, the pairwise comparison conducted on these variables showed no significant difference between the different growth habits (Fig. B5) and between plantation and ratoon crop cycles (Fig. B6).

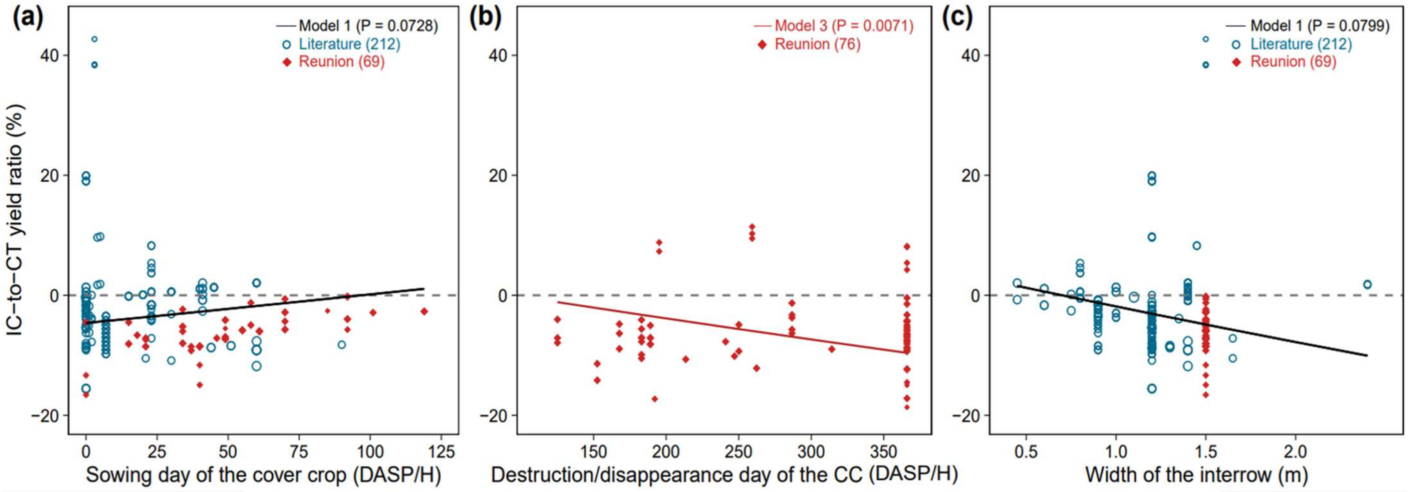


Fig. 3. The IC-to-CT yield ratio depending on (a) the legume sowing date (in days after planting or harvest DASP/H), (b) the legume destruction or disappearance date (DASP/H), and (c) the width of the inter-row (m). The black and red lines represent the fitted random-effects model regression of models 1 and 3, respectively. The blue open circles and red filled points represent the literature and Reunion predicted values, respectively. The p.value and the number of observations are indicated in parentheses.

3.5. Effect of intercropping with legume over the years

The number of years of intercropping was a significant covariate in the full data set (Model 1) and the literature data set (Model 2), but it was not selected in the Reunion data set (Table 2). In the full data set, the IC-to-CT ratio decreased by 0.7% per year of intercropping (Fig. 4).

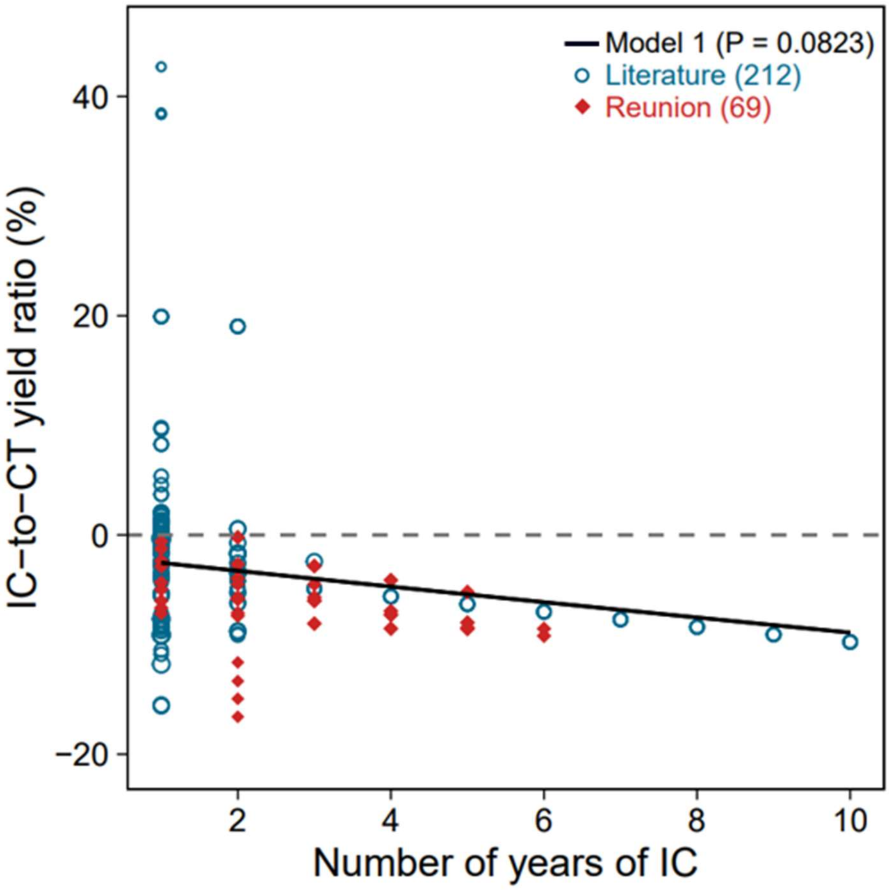


Fig. 4. The IC-to-CT yield ratio depending on the number of years since the implementation of the IC treatment (years). The black line represents the fitted random-effects model regression in model 1. The blue open circles and red filled points represent the literature and Reunion predicted values, respectively. The p.value and the number of observations are indicated in parentheses.

3.6. Legumes and weed effects on cane yield

In the Reunion data set, the IC-to-CT yield ratio decreased with the global mean ground cover (Fig. B7). The analysis showed that weeds had an effect two times stronger than legumes on sugarcane yield when respective ground covers were used in the model. The IC-to-CT yield ratio decreased with weeds' mean ground cover (slope = -0.33, Model 3), while it increased with legume mean ground cover (slope= 0.15, Model 3, Table 2, Fig. 5). Nonetheless, when using the global ground cover by both weeds and legume, the IC-to-CT yield ratio decreased with an increase in the global ground cover of the inter-row.

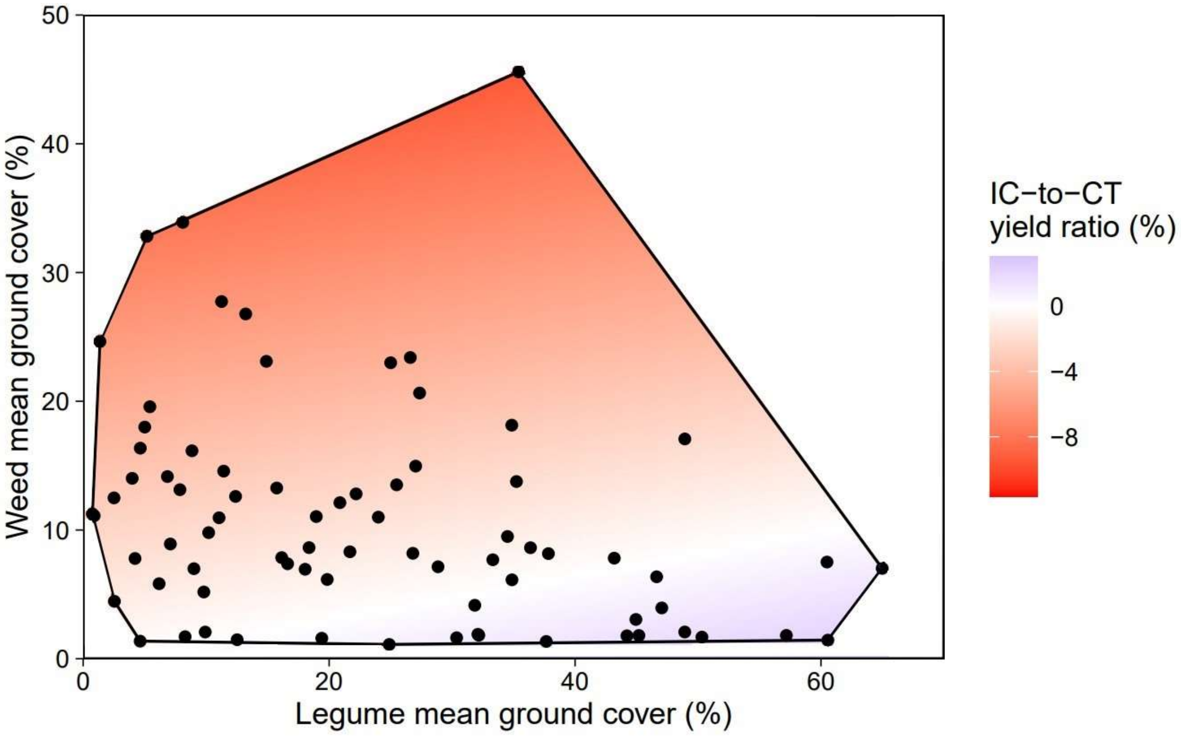


Fig. 5. The IC-to-CT yield ratio (%, red to purple gradient) depending on the ground cover by legume (x-axis) and weeds (y-axis). The IC-to-CT was predicted based on Model 3 (Reunion data set). The black line delimited the area of observed ground cover values in the Reunion data set. The black-filled points represent the Reunion predicted values.

4. Discussion

4.1. Variable effect of legume companion plants on sugarcane yield

Our meta-analysis showed that legume intercrops had on average a slightly negative effect on sugarcane yield compared to the monoculture treatment. However, the effect varied widely among the studies, negatively affecting sugarcane yield in 63% of cases. Such variability was expected considering the wide range of climate, soil, and management practices considered in this meta-analysis. These results are similar to those obtained by Verret et al. (2017) in a worldwide meta-analysis on intercropping systems with annual cash crops (mainly maize and straw cereals) and legumes. They found a negative effect of legumes on cash crop yield in 56% of cases compared to the monoculture-weeded treatment. Similar to our results, the lowest IC-to-CT yield ratio in Verret et al. (2017) reached−49%, however, they found a higher maximum positive effect reaching +317%.

The negative effect on sugarcane yield was larger in data issued from Reunion studies than in the literature. There is no apparent reason related to the experimental conditions that could explain such a difference. A publication bias may explain this difference and lead to underestimating the effect of legumes on sugarcane yield in the literature. Publication bias may be due to unpublished studies with negative results and the preferential publication of significant results compared to non-significant results (Makowski et al., 2019). We could not highlight any publication bias, but our data set did not allow us to test it properly.

4.2. Influence of resource availability in sugarcane-legume competition

Our results showed that sugarcane yield varied with resource availability related to climatic conditions and soil types. Our study highlighted how light access by the legume was an important factor in the competitive interactions between sugarcane and legume. An increase in the distance between sugarcane rows induced greater and longer light accessibility to the legume and weeds. Similar results have been shown with other crops, such as cotton, soybeans, corn, and rice, where low row spacing reduced weed density and its impacts on yield (Bradle,y 2006; Chauhan and Johnson, 2011; Tursun et al., 2016). However, row spacing can be difficult to change as it responds to local technical constraints and can only be achieved at planting time.

The present study also highlighted how legume competition was stronger at lower temperatures. Such observation is explained by the fact that sugarcane is a tropical crop sensitive to low temperatures (Mongelard and Mimura, 1972). In addition, the ratio decreased with altitude, which is also associated with a decrease in global radiation (Chopart, Mézino, and Nativel, 2003). Temperature and global radiation are key factors explaining variability in sugarcane yield on a regional scale (Christina et al., 2021b; Marin and Carvalho, 2012).

Our study also showed that the yield ratio increased with soil fertility (soil organic carbon). Similarly, Xu et al. (2020) showed an increase in the land equivalent ratio with soil organic matter.

Soil organic Nitrogen is the main source of nutrition for sugarcane through the mineralization process (Dourado-Neto et al. 2010). A higher soil N stock usually results in a higher soil N supply, reducing sugarcane N stress and interspecific competition. This statement was also supported by the yield ratio response to high temperature, which is one of the main factors controlling soil organic N mineralization. In opposition, our study did not show any effect of N fertilization on yield ratio. Nonetheless, these results are consistent with other meta-analyses on intercropping systems (Pelzer et al., 2014; Xu et al., 2020), showing that the effect of nitrogen fertilizer is not significant but appears to be highly variable, stressing the need for deeper analysis.

Finally, mean annual rainfall or irrigation did not significantly affect sugarcane-legume competition in our study. However, the sugarcane yield ratio tended to increase with rainfall in the non-irrigated experiments (Fig. B3). Several studies have shown that an increase in water availability reduces inter-species competition (Daellenbach et al., 2005; Roy et al., 2015; Waddington et al., 2007).

4.3. Trade-off in sugarcane-legume intercropping

Our study highlighted legume's sowing and destruction date as key factors driving the competitiveness of sugarcane and legume. An early sowing date of the companion plant resulted in a decrease in sugarcane yield in the present study, supporting the assumption that sugarcane is highly sensitive to competition during early growth. It can be assumed that the legume can over-compete sugarcane for light, water, and nutrients when established early. Thus, delaying the sowing of the companion plant relative to the sugarcane planting/harvest day should reduce any yield penalty. These results are in agreement with a meta-analysis on cereal-legume intercropping systems (Yu et al., 2016). Yu et al. (2016) also showed that this effect was more significant when the first sowed species was fertilized. Although no interaction between the sowing date and nitrogen fertilization was found in this study, this topic appeared as an interesting avenue to explore in future research.

The companion plant's early destruction or disappearance date also positively impacted sugarcane yield, suggesting its destruction may have facilitated sugarcane growth through an additional supply of N from the mineralization of legume residues. An expected benefit of legume intercropping is interspecific facilitation through N transfers during senescence and decomposition of the different components of legumes (aerial biomass, roots, and nodules, Yadav and Yaduvanshi 2001). Legumes with low C/N and lignin/N are quickly mineralized (Handayanto et al. 1995). In opposition, before companion crop death, sugarcane could benefit from root exudates (Thilakarathna et al., 2016) and increased resource availability (Latati et al., 2014). Indeed, higher available NPK and microbial activity have been observed in short-term sugarcane experiments (Lian et al., 2019; Solanki et al., 2019). To our knowledge, the effect of destruction date has not been addressed in intercropping systems. However, the destruction date is a major issue in cover crop-based rotational systems, influencing cover crops' beneficial or detrimental effect on the following cash crop (Keene et al., 2017; Mirsky et al., 2013). Alonso-Ayuso et al. (2014) showed that the destruction date must be adapted according to the dynamic of mineralization (linked to the residue quality and climate and soil conditions) to ensure synchronization between N release from cover crops and the cash crop N requirement. These principles can be transposed to sugarcane-legume intercropping systems. The destruction date must be considered in future work to maximize the potential benefits of legumes as green manures by enhancing soil properties (physical and chemical). Destroying the legume during the sugarcane cycle involves additional costs and labor time and can be technically complex when the sugarcane is well-developed. A solution could be to use legume varieties with short growth cycles.

The amount of N fixed by the legume will depend on agro-environmental conditions and the crop's growth (13 to 60 kg N ha-1 in the studies in this meta-analysis, de Resende et al., 2003; Tian et al., 2020). A late sowing date should benefit the sugarcane's competitiveness and productivity but at the expense of the legume's growth. One of the studies selected for this meta-analysis observed that biomass and N uptake by legumes was three-fold higher for early sowing compared to late sowing (Prellwitz and Coelho 2011). In addition, early sowing dates can facilitate legume establishment and enhance weed control (Mansuy et al., 2016). The destruction date must also allow sufficient N accumulation to maximize expected legume benefits. The timing of sowing and destruction of companion plants plays a crucial role in the performance of these intercropping systems. Further work is therefore needed to optimize legume sowing, and destruction dates to find a trade-off between expected services (N enrichment and weed control) and disservices (yield loss, costs, and labor time).

4.4. Sustainability of sugarcane-legume intercropping

Our study showed that the IC-to-CT yield ratio decreased over time contrary to previous results in other intercropping systems. For example, in long-term maize-wheat and maize-legume experiments, Li et al. (2021) observed an increase in the IC-to-CT yield ratio and attributed this feature to an increase in soil fertility over time.

A positive effect of intercropping on soil fertility is expected, through an enhancement of the soil organic C and N content (e.g., in temperate climates, Cong et al., 2015; Dybzinski et al., 2008). Nevertheless, the only study dealing with the long-term effect of legumes on soil properties in sugarcane-legume intercropping systems did not find an increase of soil organic carbon content (e.g., in China, Wang et al., 2020). Carbon and Nitrogen inputs from legumes into the soil will depend on the quantity and quality of residues and their interaction with the environment. In sugarcane intercropping systems, the produced biomass and fixed N are relatively low compared to legumes as a rotation break crop (de Resende et al., 2003; Shoko et al., 2008). Such a low amount of C combined with low soil C stabilization ability (Feller and Beare, 1997) and higher mineralization rate in tropical conditions (Ogle et al., 2005; Tiessen, 1998) may explain why the effect of legume on soil C was less noticeable than in temperate conditions.

The amounts of nitrogen supplied by legumes can be significant, but the risk of loss could be high under tropical conditions. Indeed, the good decomposability of legume residues and the high mineralization rate in the tropics can result in massive emissions of mineral N. A growing number of studies point to losses of N from legumes through ammonia volatilization (Glasener and Palm, 1995; Nett et al., 2016), N_2O emissions (Peyrard et al., 2016), and nitrate lixiviation (Quemada et al., 2013). Ammonia volatilization, favored by sugarcane mulch (Pinheiro et 2018) can be limited by burying crop residues. Still, attention must be paid to subsequent nitrate losses by leaching in a tropical context characterized by heavy rainfall. The fate of carbon and nitrogen needs to be studied further to assess the effect of intercropping legumes on soil fertility under tropical conditions. Nonetheless, a modification of soil fertility, as limited as it may be under the tropics, cannot explain the sugarcane yield decreases observed over time in the present study.

To explain the decrease of the IC-to-CT yield ratio over the years, a second explanation is that the negative impact of legumes on one year could be passed on onward. Indeed, a negative effect on sugarcane growth can reduce the belowground reserves at harvest and thus slow down the growth of the following cycle. This hypothesis must be nuanced because many experiments (mainly in Asia) in the literature replant sugarcane yearly. However, we observed the same trend when restricting the data set to only the experiments that did not replant each year (Fig. B8). Additionally, weed control by companion plants is limited in intercropping compared to chemical treatment (Verret et al., 2017). Therefore, a third explanation could be an increase in weed pressure over time compared to conventional systems (observed in one long-term experiment in Reunion Island, Experiment 2). Our analysis showed that the IC-to-CT yield ratio decreased with the global mean ground cover (legumes and weeds). However, we also showed that weeds were more damaging than companion legumes to sugarcane yield. This result can be attributed to lower competition for N of the legume due to their ability to fix N from the atmosphere. In addition, multi-species communities like weeds have a higher potential competitiveness because species have a range of resource acquisition strategies. Consequently, the sugarcane–legume–weeds interaction must be further investigated to optimize the long-term sustainability of sugarcane-legume intercropping. Additionally, a greater diversity of legumes could be evaluated in sugarcane intercropping systems, particularly legume mixtures that have shown good potential to limit weed growth (Baraibar et al., 2018).

5. Conclusion

Despite an average 3.34% decrease in yield in the sugarcane-legume intercropping system compared to monocropping, this meta-analysis supports the assumption that legumes are less damaging than weeds for sugarcane, consolidating sugarcane-legume intercropping as a promising practice to reduce herbicide use. The effect of legumes intercrops on sugarcane yield was significantly influenced by environmental conditions (e.g., annual temperature, soil organic carbon content) and management practices (e.g., sowing and destruction dates), allowing to suggest some management options to limit the negative impact of legumes on sugarcane yield. However, there were currently insufficient elements to reason the management practices regarding different environmental conditions. There is a need for additional studies across various environments and situations to unlock the potential of sugarcane-legume intercropping and to encourage the spread of this technique among farmers. Finally, very few studies have quantified the amount of N provided by legume fixation. Further research should investigate the long-term benefits on soil fertility of legumes in intercropping systems with sugarcane.

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Author contributions

P. Viaud: Conceptualization, Methodology, Investigation, Formal analysis, Writing - Original Draft. B. Heuclin & P. Letourmy: Methodology, Formal analysis, Visualization. M. Christina, A. Versini & K. Naudin: Conceptualization, Methodology, Validation. A. Mansuy & J. Chetty: Conceptualization, Investigation. All authors: Writing - Review & Editing.

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Chapter 2: Sugarcane x Canavalia ensiformis competition in intercropping system under contrasted nitrogen and water availability

Ce travail a été réaliser sur un essai expérimental sur la station de La Mare mise en place par l'équipe PROSE de l'unité AIDA à La Réunion. Les personnes impliquées ainsi que leurs contributions respectives à ce chapitre de thèse sont listées ci-dessous.

Pauline Viaud a effectué le suivi de l'essai, la gestion des échantillons, l'analyse des données et la rédaction du chapitre. **Mathias Christina** a mis en place et suivi l'essai, participé à l'analyse des données et à la rédaction du chapitre. **Antoine Versini** a apporté la méthodologie pour l'estimation de la biomasse de la canne en cours de croissance et le suivi de l'azote du fertilisant dans les microplots ^{15}N, à l'analyse des données et à la rédaction du chapitre. **Krishna Naudin** a participé à l'interprétation des résultats et à la rédaction du chapitre. **Julie Hoareau** et **Amandine Laurent** ont participé au suivi de l'essai et aux traitements des échantillons durant leur alternance en 3ème année de Licence Professionnelle de l'Université de La Réunion. **Léa Chevalier** et **Sophie Graillot** ont collecté et traité les échantillons de racines durant leurs de stage de fin d'étude d'école d'ingénieur (SupAgro Montpellier). **Jean-Maurice Gueno**, **Ginot Gauvin**, **Jean-Claude Ribotte**, **Emmanuel Hoarau** et **Willy Hoarau**, constituant l'équipe de technicien et d'ouvrier de l'unité AIDA ont apporté leur appui dans la mise en place de l'essai, gestion de l'itinéraire technique et prélèvements des échantillons sur le terrain. L'équipe technique de eRcane a également apporter son appui lors des semis des légumineuses. **Marion Collinet** et **Jocelyn Idmond** ont réalisé le dosage d'azote total des échantillons de canne-à-sucre et de légumineuse au laboratoire Cirad de La Réunion. **Christian Hossann** a réalisé les analyses d'azote ^{15}N au laboratoire PTEF de l'INRAe de Nancy.

Abbreviations

CS	Cropping system
SC	Sugarcane
CP	Companion plant
MAH	Months after sugarcane harvest
MAS	Months after companion plant sowing
TVD	Top visible dewlap
NdfF	Nitrogen derived from fertilizer
NRE	Nitrogen recovery efficiency
ADM$_{SC}$	Sugarcane aboveground dry mass
ADM$_{CP}$	Companion plant aboveground dry mass
Root_DM$_{SC}$	Sugarcane root dry mass
Nb_Till$_{SC}$	Number of tillers
Height$_{SC}$	Height TVD of sugarcane
Stalk_P$_{SC}$	Stalk proportion of sugarcane
Root_P$_{SC}$	Root proportion of sugarcane
Yield$_{SC}$	Sugarcane yield
Lodging$_{SC}$	Sugarcane lodging
SEM	Structural equation model

1. Introduction

Intercropping is usually defined as cultivating two or more crops in the same space for a significant part of their growing periods (Willey, 1979). Intercropping has been promoted in many cropping systems to provide various benefits simultaneously, such as higher land utilization efficiency (Martin-Guay et al., 2018), a non-chemical pest and weed control management method (Gardarin et al., 2022), and an increase in soil fertility (Wang et al., 2014; Yu et al., 2022). In particular, intercropping with legumes can improve soil nitrogen (N) availability and N nutrition of the non-legume species (Guiducci et al., 2018).

Low N-use efficiency and environmental impacts of N losses are major issues in sugarcane cropping (Thorburn et al., 2017; Wang et al., 2020). In addition, weeds are among the major constraints of sugarcane cultivation. Therefore, the introduction of legume companion plants in sugarcane systems is an interesting alternative to minimize the requirements for N fertilization and herbicide (Dantas et al., 2015; de Resende et al., 2003). However, introducing a companion crop can lead to undesirable competition for light and soil resources and can result in a trade-off between agronomic and environmental performances (Justes et al., 2021; Thorsted et al., 2006; Verret et al., 2017). We showed in a meta-analysis that intercropping with legumes resulted in lower sugarcane yields compared to the monoculture treatment in 63% of cases (Viaud et al., 2023).

Sugarcane yield is the fresh stalk mass (which contains sugar) harvested before the flowering stage. Consequently, the observed decrease in yield in previous studies may result either from a reduction of overall biomass in the plant or from a change in biomass allocation between its different compartments (above and belowground parts, as well as tillering). While the change in overall biomass may result from resource competitions (e.g., water and soil nutrients), the shift in biomass allocation may result from plant plasticity, as sometimes observed in other intercropping systems (Bargaz et al., 2017). These two aspects have been poorly investigated in sugarcane intercropping systems.

Sugarcane-legume intercrops include a tall (i.e., sugarcane) and a short (i.e., legume) plant. Thus, competition for light should be limited concerning sugarcane, and sugarcane growth is more likely affected by below-ground competition for water and nutrients. In a meta-analysis, Viaud et al. (2023) showed that resource availability influenced sugarcane yield response to legume intercropping. In particular, inter-species competition was reduced with increasing soil fertility. Regarding water competition, Viaud et al. (2023) did not identify a significant effect of rainfall and irrigation in sugarcane intercropping systems. However, several studies on other crops have shown that increased water availability reduces inter-species competition (Daellenbach et al., 2005; Roy et al., 2015; Waddington et al., 2007). To limit competition, a meta-analysis on cereal-legume intercropping systems showed that the cash crop's fertilization before the companion plant sowing provides a competitive advantage to this crop (Yu et al., 2016). Indeed, the beginning of sugarcane growth (0-4 months) will be a crucial period in terms of competition as it corresponds to the period with the highest growth rate and demand for nutrients (dos Santos Wanderley et al., 2021; Tadele et al., 2022).

In the context of intercrops, the environment experienced by a crop is linked to local soil and weather conditions, as well as field management, including the establishment of the other plant species as part of the mixture (Gebru, 2015). Consequently, trait and biomass allocation plasticity has been observed in different cash crops (e.g., cereals and soybean, Ajal et al., 2022; Hussain et al., 2020). In some cases, plasticity, notably for root systems, may even contribute to crop recovery and limit the impact on yield (Liu et al., 2020; Zhang et al., 2022). While the sugarcane plasticity in response to intercropping has been poorly investigated (e.g., Takaragawa et al., 2018), sugarcane biomass allocation to roots is known to be influenced by environmental conditions such as soil type, soil nitrogen, and water availability (Chevalier et al., 2023; Versini et al., 2020; Yang et al., 2022). Additionally, sugarcane's tillering phase is highly sensitive to environmental conditions (Gomathi et al., 2015; Zhao et al., 2014) and could thus be influenced by intercropping, as shown in other cropping systems (Ajal et al., 2022).

This study aimed to assess the competition mechanism in a sugarcane x legume (*Canavalia ensiformis* (L.) DC (Jack bean)) intercropping system depending on environmental conditions (water and nitrogen availability). To this aim, a three-year experiment has been set up to test the interactions between irrigation, nitrogen fertilization, and legume intercropping. The competition for soil N and N from fertilizer was distinguished. In parallel with the experiment, the competition for the N of the fertilizer was investigated using ^{15}N-labelled fertilizer in microplots during one cycle. The hypotheses were:

i. The change in sugarcane yield, due to intercropping, would result from a decrease in overall biomass and a shift in biomass allocation pattern.
ii. The sugarcane-legume competition would be higher at the beginning of the sugarcane growth during the period with the highest sugarcane demand for nutrients and water.
iii. The impact on sugarcane would result from water and soil nitrogen competition under constrained conditions.

2. Methods

2.1. Study site, climate, and soil characterization

This study was carried out on the research station of La Mare, located on the northern coast of Reunion Island (20°54 S, 55°31 E, 69 m a.s.l.) over three cropping seasons (i.e., three years) in ratoon sugarcane. The climate was tropical, with a rainy season from January to March and a dry season from May to November. The mean annual rainfall was approximately 1800 mm, and the mean annual temperature was 24 °C (means over 10 years). The soil of the experimental site was a Hypereutric Nitisol according to the WRB classification (USS Working Group WRB 2015) with a silty loam texture. Daily climatic data were measured in a nearby Campbell meteorological station (50 m from the trial) and obtained from the Meteor software from Cirad (https://smartis.re/METEOR). Soil chemical characteristics were determined before the sugarcane plantation (06/2018, Table 1). The soil was sampled at three depths (0-20, 20-40, and 40-60 cm) and 9 positions in the trial and pooled by depth. Total N was analyzed with the Dumas method, and total P with the Olsen-Dabin method.

Table 1. Chemical and physical characteristics of the soil at the beginning of the trial in 2018*

Soil layer (cm)	pH_{water}	Total N $g\ kg^{-1}$	Corg $g\ kg^{-1}$	Total P $mg\ kg^{-1}$	CEC $cmol\ kg^{-1}$	Clay %	Silt %	Sand %	Bulk density $g\ cm^{-3}$
0-20	6.10	1.92	21.33	115.70	12.16	16.4	54.9	28.7	1.16
20-40	6.25	1.36	14.20	61.24	10.88	17.7	55.6	26.7	1.15
40-60	6.41	0.73	7.34	51.81	9.43	55.6	34.3	10.1	1.29

*: Clay, Silt, and Sand% were determined during a previous study in 2015. (Jones et al., 2019; 2021)

2.2. Experimental design

The trial included eight treatments replicated in four blocks (Figure 1). The experiment consisted of a factorial trial with three crossed treatments:

- A cropping systems treatment (hereafter CS treatment) with two levels: sugarcane x companion plant, *C. ensiformis*, intercropping (SC + CP) or sugarcane monocropping (SC)
- A nitrogen fertilization treatment (hereafter N treatment) with two nitrogen levels N (0N: 0 kg N ha^{-1} and Nopt: 137 kg N ha^{-1} as optimal/recommended nitrogen application level),
- An irrigation treatment (hereafter W treatment) with two levels (rainfed 0W and irrigated W with sprinklers). The rainfed treatment was irrigated the first two months after previous harvest to ensure initial sugarcane and companion plant growth.

The trial was divided into two parts (rainfed or irrigated). Cropping systems and nitrogen levels were randomly nested based on a Latin hypercube sampling within these two parts. Each elementary plot consisted of height rows of 11 m of sugarcane with a spacing of 1.5 m between rows (Figure 2). Eight modalities were tested: W+Nopt+SC+CP, W+Nopt+SC, 0W+Nopt+SC+CP, 0W+Nopt+SC, W+0N+SC+CP, W+0N+SC, 0W+0N+SC+CP, 0W+0N+SC.

Figure 1. Aerial photography and experimental design of the trial. 0W/W: Rainfed / Irrigated ; 0N/Nopt: 0 kg N ha^{-1} / 137 kg N ha^{-1} ; SC/SC + CP: Sugarcane monocropping / Sugarcane x Companion plant intercropping. The violet rectangles refer to plots with microplot ^{15}N urea. The filled blue dotes indicated permanent irrigation with sprinklers, while the blue circle indicated temporal irrigation for the first two months after the sugarcane of harvest.

2.3. Crop management

Sugarcane cultivar R579, adapted for wet and low-altitude areas, was planted in September 2018 with 18 nodes per meter density and a 1.5 m inter-row. The sugarcane crops were harvested in October every year, and crop residues (sugarcane straw) were left on the field. The measurements in this study were conducted during the first, second, and third ratoon crops.

C. ensiformis was selected as a legume companion crop because it has been identified as a great candidate to control weeds without over-compete with sugarcane in previous studies (Christina et al., 2021b; Mansuy et al., 2019). *C. ensiformis* was sown manually each year with a density of 58 kg ha^{-1}, 1.5 months after sugarcane harvest on two rows spaced 40 cm apart each over and 55 cm apart sugarcane rows. Legume companion crops were not destroyed but gradually disappeared due to sugarcane canopy closure (around eight months after harvest).

All plots were manually fertilized on the sugarcane row with 42 kg P ha^{-1} and 190 kg K ha^{-1} one month after the harvest. The nitrogen fertilizer rate (137 kg N ha^{-1}) was split into two equal applications, one month and three months after the sugarcane harvest (November 2019/2020/2021 and January 2020/2021/2022). No fertilizer was applied on the inter-row. The W treatments were irrigated with a sprinkler system throughout the crop cycle (around 19 mm $week^{-1}$) except for the last two months before harvest to favor the ripening of sugarcane. The total water supplied via irrigation was 1010, 777, and 738 mm for the ratoons 1, 2, and 3, respectively.

The 0W treatments were irrigated with a sprinkler only during the first two months after the sugarcane harvest.

The trial was managed to prevent weed development. A pre-emergence herbicide was applied after sugarcane planting or harvesting and before *C.ensiformis* sowing. Afterward, weeding interventions were performed when weeds reached 30% of soil ground cover (chemically for SC plots and manually for SC+CP plots). Details on fertilization and herbicide application dates are given in Tables 2 and 3.

Table 2. Details of the fertilization management

Sugarcane cycle	Date	Treatment	N kg ha^{-1}	P kg ha^{-1}	K kg ha^{-1}	Fertilizer type
Ratoon 1	06/12/2019	All		35	160	Physalg27 (0-27-0); Sufate K (0-0-50)
	09/12/2019	Nopt	68,5			Urea (46-0-0)
	24/01/2019	Nopt	68,5			Urea (46-0-0)
Ratoon 2	24/11/2020	All		42	190	MKP (0-52-34); Sufate K (0-0-50)
	24/11/2020	Nopt	68,5			Urea (46-0-0)
	10/02/2021	Nopt	68,5			Urea (46-0-0)
Ratoon 3	29/11/2021	All		42	190	MKP (0-52-34); Sufate K (0-0-50)
	29/11/2021	Nopt	68,5			Urea (46-0-0)
	26/01/2022	Nopt	68,5			Urea (46-0-0)

Table 3. Details of weeds management

Sugarcane cycle	Date	Treatment	Type	Description and doses*
Ratoon 1	30/10/2019	All	Herbicide	Sencoral (1 L ha^{-1}) + Callisto (1 L ha^{-1}) + 2,4-D (1 L ha^{-1})
	04/12/2019	SC	Herbicide	Starane (1.5 L ha^{-1}) + Dicopur (1.5 L ha^{-1})
Ratoon 2	26/11/2020	All	Herbicide	Camix (4 L ha^{-1}) + Merlin (30 g ha^{-1}) + Prowl (3 L ha^{-1})
	26/01/2021	All	Manual	Poaceae and twining species
	03/02/2021	SC	Herbicide	Dicopur 600 (2 L ha^{-1}) + Garlon (1 L ha^{-1}) + Camix (1 L ha^{-1}) + Roundup (1 L ha^{-1})
	09/02/2021	SC	Manual	Poaceae
Ratoon 3	25/11/2021	All	Herbicide	Callisto (1 L ha^{-1}) + Dicopur (2 L ha^{-1})
	18/01/2022	All	Manual	Poaceae
	30/03/2022	SC + CP	Manual	Twining species

*herbicides composition: Sencoral (600 g L^{-1} metribuzin), Callisto (120 g L^{-1} dicamba 50 g L^{-1} mesotrione), Dicopur (600 g L^{-1} 2,4-D), Starane (200 g L^{-1} fluroxypyr), Camix (20 g L^{-1} Bénoxacor, 400 g L^{-1} S-metolachlore, 40 g L^{-1} mesotrione), Merlin (44 g L^{-1} isoxaflutole, 44 g L^{-1} cyprosulfamide), Prowl (400 g L^{-1} pendimethalin), Garlon (240 g L^{-1} triclopyr, 30 g L^{-1} aminopyralide), Roundup (450 g L^{-1} glyphosate acid).

2.4. Field measurements

2.4.1 Leaf area, radiation interception, soil water content

Radiation interception was measured using a SunscanTM canopy layer each month in three positions: above the sugarcane canopy, above the companion plant, and on the soil. In each plot, six measurements with different positions were made (Figure S1). The total leaf area index (LAI) of the sugarcane alone or sugarcane and legume was calculated by the Sunscan. Soil water content (SWC $cm^3\ cm^{-3}$) at depths 10, 20, 30, 40, 60, and 100 cm was measured using a frequency domain reflectometry sensor (PR2 – Profil Probe, Delta-T Devices Ltd., Cambridge, UK) each week during the first ratoon. Due to technical problems, we could not conduct this measurement in the following cycles. Measurements were made in one tube in the soil per elementary plot located at a 40 cm distance from the sugarcane row. Between each measurement depth and at each date, SWC was linearly interpolated, and averaged values were calculated for soil layers: 0-25 cm, 25-50 cm, and 50-100 cm.

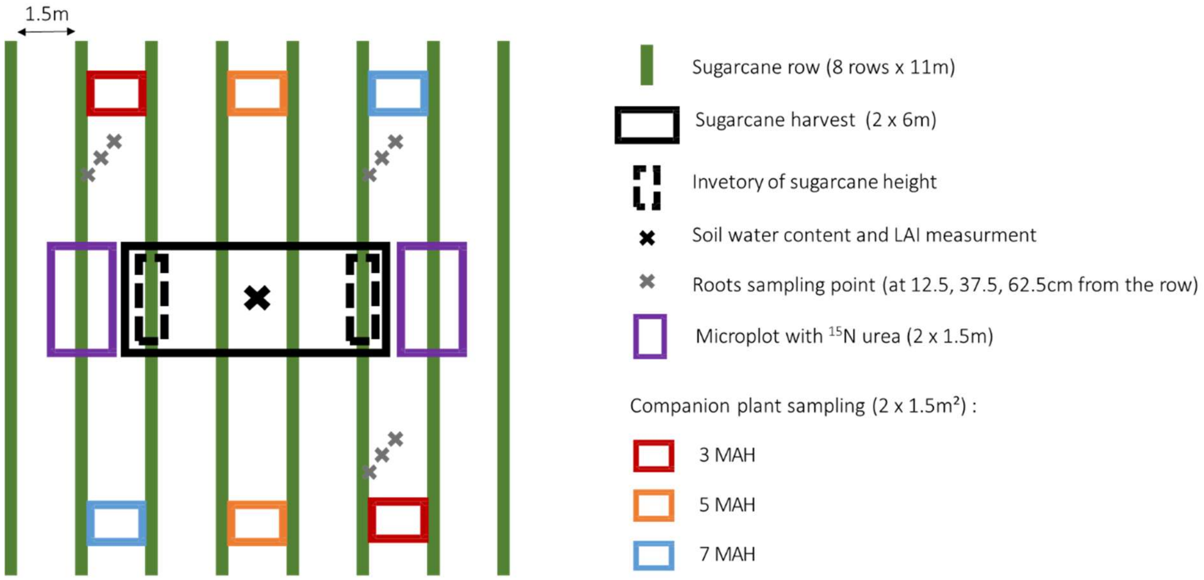

Figure 2. Schematic representation of a plot with sampling zones.

2.4.2 Aboveground sugarcane biomass, N content, N nutrition index, and number of tillers

The aboveground biomass and N content of sugarcane was measured during the growth cycle at 3, 5, 7, and 12 months (January, March, May, and October) after the previous sugarcane harvest (MAH, Figure 2).

During the crop cycle (at 3, 5, and 7 months), the aboveground biomass of sugarcane (t ha^{-1}) was estimated in each plot using an allometric relationship method between height and biomass of sugarcane to avoid destructive samplings (Poultney et al., 2020). Forty individual sugarcanes were sampled at each date, equally distributed among the different treatments. The height of each sugarcane was measured from the base cut to the top visible dewlap leaf (height TVD). The dry biomass of the 40 sugarcanes was weighed after they were oven-dried at 60 °C for 72 h. To build the allometric model between sugarcane aboveground biomass and height, several models were compared with the AIC criteria (Akaike Information Criterion). A power function across sampling dates and treatments was retained to construct an allometric relationship between sugarcane biomass and height for each ratoon cycle (Figure S2, Table S1). To estimate the sugarcane biomass of each plot, inventories of sugarcane height TVD were made on the same dates. Sugarcane height TVD was measured for each sugarcane stalk on 2 rows of 2 meters in the center of each plot (Figure 2). The biomass of each sugarcane was estimated from the allometric relationships; then, sugarcane aboveground biomass was extrapolated at the plot scale. The height inventories were also used to calculate the number of tillers per m^2 in each plot.

At the sugarcane harvest (12 MAH), all aerial tissues were collected from each plot's four central rows x 3 m and freshly weighted. Then, three sugarcane samples were divided into the stem, green, and senescent leaves and freshly weighed. A sub-sample of each component was oven-dried at 60°C for 72h and weighed to obtain dry matter content. The stalk fresh mass at harvest was defined as the sugarcane yield.

Sub-samples of 3 sugarcane at each date (during the crop cycle and at harvest) in each plot were taken to measure the N content using an elemental analyzer (Vario Max Cube, Elementar Analyse Systeme GmbH, Hanau, Germany). The nitrogen nutrition index (NNI) was determined using the concept of critical nitrogen concentration and nitrogen dilution curves (Greenwood et al., 1991) where the NNI is the ratio between the actual nitrogen content and the critical nitrogen content for the same aboveground dry biomass. The critical nitrogen dilution curves for sugarcane in Reunion Island was obtained from Pouzet et al. (1999).

2.4.3 Aboveground legume biomass and N content

In each plot, the aboveground biomass of *C. ensiformis* was estimated from 2 samples of 1.5 m^2 at each sampling date (3, 5, and 7 MAH, Figure 2). A sub-sample was oven-dried at 60°C for 72h and weighed to obtain dry matter. Around 200g of companion plants were tokens to determine the N content.

2.4.4. Belowground sugarcane biomass

Sugarcane root biomass was measured at 12 months during the 1st, 2nd, and 3rd ratoon and at 6 months during the 2nd and 3rd ratoon. At 6 months, three 0-50 cm cores were sampled in the middle of each plot at 12.5, 37.5, and 62. cm from the sugarcane row, using a mechanical auger – Cobra TT thermal core with an auger of 9 cm diameter following the methodology of (Chevalier et al., 2023). At 12 months, only two core samples were measured (12.5 and 62.5 cm), assuming that the 37.5 cm position would have the same root biomass density as 6 months (based on previous studies, Versini et al., 2020). All soil cores were washed using a 1 mm sieve to extract sugarcane roots. At 6 months, sugarcane and legume roots were visually separated. Legume roots were less reached and lighter in color than the sugarcane roots, making it possible to distinguish them. Sugarcane roots were dried at 60°C for 72 hours to obtain root dry mass.

2.4.5. Sugarcane lodging

In June of each year (8 months old), the number of lodged sugarcane tillers was measured in each plot excluding the two border rows. A tiller was characterized as lodged when its angle was approximately less than 45°C with the ground.

2.5. ^{15}N-labeling experiment

2.5.1. Microplots for the ^{15}N-labeling experiment

The competition for the N fertilizer between sugarcane and the companion plant was studied using ^{15}N-labeled urea. The two urea applications were separated to evaluate the competition between sugarcane and legumes according to the urea application dates. This experiment was conducted on one crop cycle during the first ratoon (2019-2020) in the treatments W + Nopt + SC and W + Nopt + SC+CP. Two microplots of 3 m^2 (2 m x 1.5 m) were installed inside 3 plots of each treatment (see Figure 1), based on Trivelin et al. (1994). One of the microplots received ^{15}N-labeled fertilizer (3 atom % ^{15}N excess) at the first urea application (1 month after the SC harvest / 0.5 months before CP sowing) and the other at the second (3 months after the SC harvest / 1.5 months after CP sowing). The ^{15}N fertilizers were in solution form to obtain a uniform application and manually applied on the sugarcane rows in the same quantity and at the same time as the conventional non-labeled urea. A PVC ring per sampling date was set up next to the microplots (Figure 3) for soil and mulch sampling to minimize the variability of ^{15}N abundance measurements due to mulch and soil heterogeneity. The PVC rings had a diameter of 20 cm and a height of 10 cm. The same amount of mulch was applied to each PVC ring on the day of the ^{15}N-labeled fertilizer application. The amount of mulch was determined from the mean in the two treatments studied when the microplots were set up. The ^{15}N fertilizer was applied manually on the mulch.

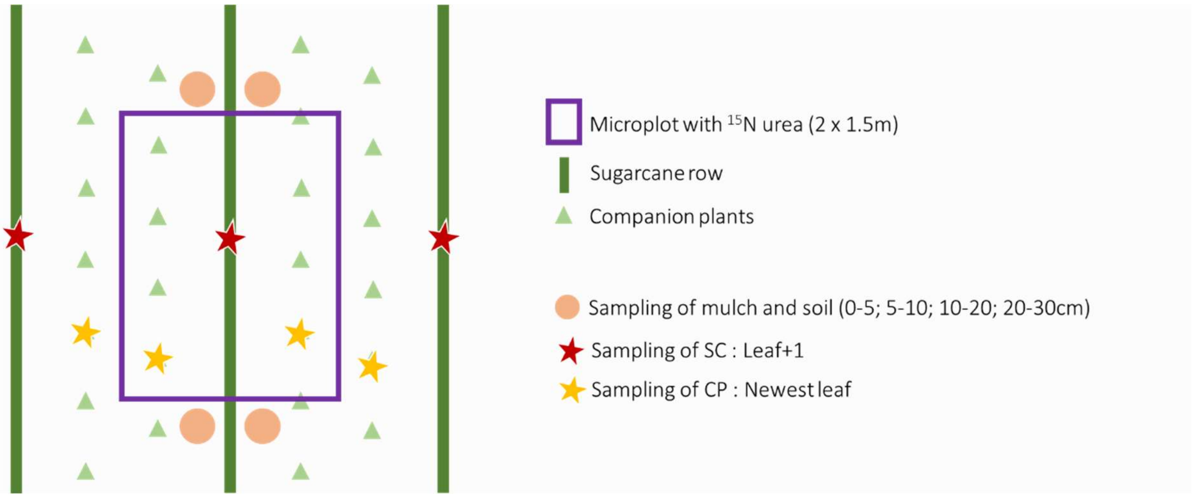

Figure 3. Schematic representation of a microplot and sampling zones.

2.5.2. .Soil, mulch, and plant sampling and analyses

The ^{15}N signature of soil, mulch, and plants was estimated at 4 dates for the first dose (at 3, 5, 7, and 12 MAH) and 3 dates for the second dose (at 5, 7, and 12 MAH). After collecting the mulch from one mesocosm, the soil was sampled at four different depths: 0-5, 5-10, 10-30, and 30-50 cm. The 0-5 and 5-10 cm soil layers were excavated, and the 10-30 and 30- 50 cm soil layers were sampled with a manual auger.

The leaf+1 (first leaf below the TVD) represented ^{15}N abundance in the sugarcane plant (Poultney et al., 2020). Leaves+1 were collected and pooled from two central sugarcane stalks of each microplot. As the neighboring plants absorb ^{15}N applied to the microplot (Trivelin et al., 1994), two leaves +1 were collected and pooled from the adjacent rows beside the microplot (Figure 3). Three individual sugarcane shoots were collected at the center of each microplots at harvest to verify the reliability of leaf+1 to represent the ^{15}N abundance in the sugarcane plant. The newest leaves were collected and pooled for the companion plant from two individuals in the center of each microplot and two individuals outside the microplot (Figure 3). For each compartment (i.e., soil, mulch sugarcane, companion plant), an unlabeled control sample was sampled with the same protocols to determine the natural abundance.

All samples were dried and ground into a fine powder in a ball mill (Cyclotec CT Sample Mill, Foss Tecator, Hillerod, Denmark). Total N concentrations (% of dry matter, DM) and ^{15}N isotopic abundance (atom%) of soil, mulch, and plants samples were measured with an elemental analyzer (Vario ISOTOPE Cube, Elementar, Langenselbold, Germany) coupled to a gas isotope ratio mass spectrometer (IsoPrime 100, Elementar UK, Cheadle, UK). Analyses were conducted at the SilvaTec platform (UMR Silva, INRA Grand Est-Nancy, France).

2.5.3. Nitrogen derived from fertilizer

The proportion of N derived from fertilizer (NdfF) of the considered compartment was determined by the following formula:

$$NdfF = \left[\frac{a-b}{c-d}\right] * 100 \qquad (1)$$

Where a is the abundance of ^{15}N atoms of the considered compartment (i.e., soil, mulch sugarcane, companion plant) (%), b is the natural abundance of ^{15}N atoms in an unlabeled control sample (%), c is the abundance of ^{15}N atoms % in the fertilizer (%), and d is the natural abundance of ^{15}N atoms of air (i.e., 0.3663 atom%). The NdfF of sugarcane and companion plant was calculated as follows:

$$NdfF_T = NdfF_M + 2 * NdfF_A \qquad (2)$$

where $NdfF_T$ is the sum of the NdfF determined from the center of the microplot ($NdfF_M$) and the adjacent plants beside the microplot ($NdfF_A$) (Poultney et al., 2020).

2.5.4. Nitrogen Recovery Efficiency

The nitrogen recovery efficiency (NRE) in the considered compartment was determined by the following formula:

$$NRE = \frac{NdfF_x * N_x}{N_{fertilizer}} \qquad (3)$$

Where $NdfF_x$ is the proportion of N in the considered compartment derived from fertilizer (%), N_x is the amount of N in the considered compartment (kg N ha^{-1}), and $N_{fertilizer}$ is the quantity of N applied with the fertilizer (kg N ha^{-1}). N_x was calculated from the N concentration in the compartment and its dry mass (kg ha^{-1}). For sugarcane, the N concentration considered was the mean N concentration of the aboveground biomass for the corresponding treatment, determined at the same time on the whole plot. The N concentration of the sample collected for this experiment was used for the over compartments. The mean dry mass of the treatment at the corresponding sampling time was used for mulch, sugarcane, and companion plant (Table S2). The mass of the soil per layer was determined by multiplying its volume with the bulk density.

2.6. Statistical analysis

Plants variables, SWC, fPAR, NdfF, and NRE, were subjected to analysis of variance (ANOVA) with linear mixed effects models. The models included the plot as a random effect and irrigation treatment, nitrogen treatment, cropping system treatments, ratoon cycle, sampling date, and their interactions as fixed effects. Insignificant interactions were eliminated for each variable until best-fitting models were formed using the Akaike and Schwarz information criteria (AIC and BIC). A post hoc analysis of each mixed linear model was then realized using Tukey's HSD test. Box-Cox transformations were used when normality and variance assumptions were not respected. All analyses were performed in R 4.1.1 (R Core Team, 2021) using packages nlme (Pinheiro et al., 2021) and emmeans (Lenth et al., 2018) for tests of linear mixed effects model fits and for post hoc tests.

Two structural equation models (SEM) were used to explore the pathways of how legume companion plants influenced sugarcane yield and lodging. In the first model on sugarcane yield, the explanatory variables were the companion plant biomass, sugarcane aboveground dry mass, root biomass, stem and root proportion, height, number of tillers, and lodging. All the explanatory variables were measured at 5 MAH during the companion plant's peak biomass, except for lodging (7 MAH). All the data were used to build the model on sugarcane yield. A second SEM on sugarcane lodging was built using only data from the irrigated plots, as it only significantly occurred in these plots. For both SEM, complete models of all possible paths were considered at the beginning, and then the insignificant paths were eliminated in turn until best-fitting and stable models were formed. Models' fitness was evaluated by χ^2 test ($p > 0.05$), Akaike information criteria, and root-mean-square error of approximation (RMSEA). SEM were conducted with the lavaan package (Rosseel, 2012) in R.

3. Results

3.1. Climate

Weather conditions during the experimental period are shown in Figure 4. The sums of temperatures reached 8897, 8437 and, 8514 degrees days in ratoon 1, 2 and, 3, respectively. Cumulative solar radiation was similar between the three ratoon cycles (6806, 6808, and 6726 MJ m^{-2} during ratoon 1, 2, and 3, respectively). Rainfall regimes were variable over the years. Ratoon 1 was characterized by a regular rainfall distribution with a slightly dry period at the end of the cycle (mainly after June: 8 MAH). Ratoon 2 was characterized by an irregular rainfall distribution with a dry period at the beginning of the cycle and some irregularity at the end of the season (in April-May and August: 6-7 and 10 MAH). Ratoon 3 was characterized by a more irregular rainfall distribution with very heavy rain during the cyclonic period (January to April: 3 MAH to 6 MAH) and a dry period at the sowing time of the companion plant (in December: 1.5 MAH) and some irregularity after April (6 MAH).

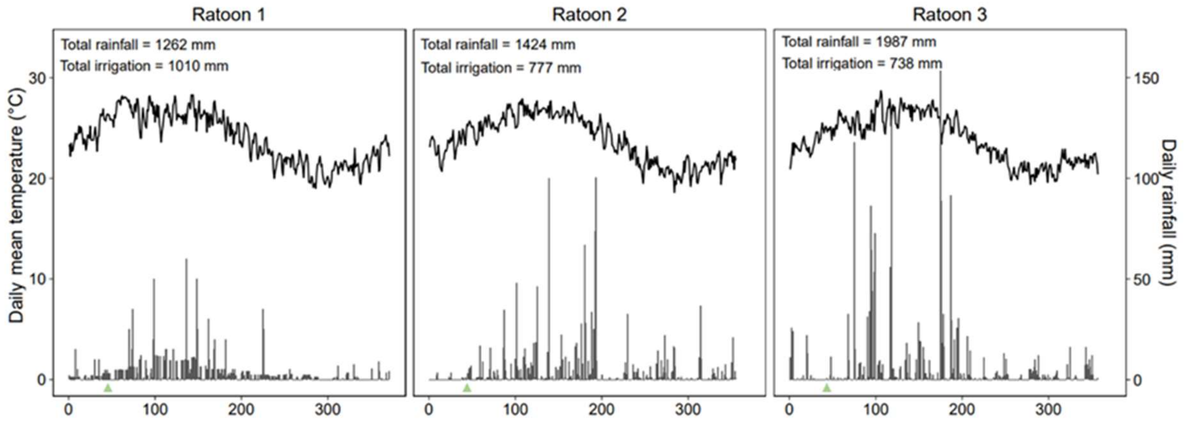

Figure 4. Daily rainfall (mm) and temperature (°C) during Ratoon 1: 2019-2020, Ratoon 2: 2020-2021, and Ratoon 3: 2021-2022 at the experimental station of La Mare, Reunion. The green triangles indicate the sowing day of the companion plant.

3.2. Sugarcane and legume growth dynamic

The ground cover by sugarcane and legume rapidly increased after harvest, with the LAI reaching 10.4 m^2 m^{-2} at 5.2 MAH (Figure S3). This rapid growth rate was also observed for the sugarcane aboveground dry mass (ADM_{SC}), with differences in the dynamics among the different treatments and the three ratoons (Figure 5a). At the beginning of the crop cycle, the growth rate of sugarcane was higher in Ratoon 1, followed by Ratoon 3, than in Ratoon 2 for all treatments. These results were related to low rainfall at the beginning of sugarcane growth in Ratoons 2 and 3 (Figure 4). In average across years and treatments, ADM_{SC} reached 47.3 and 49.9 t ha^{-1} 7 months after harvest and at harvest, respectively. The influence of the interaction between CS, N, and W treatment on sugarcane aboveground dry mass will be detailed in the following section. The aboveground dry biomass of the companion plant (ADM_{CP}) varied widely among treatments and ratoon cycle, with a range from of 0.64 to 3.21 t ha^{-1} at flowering stage (5 MAH i.e., 3.5 months after CP sowing, Figure 5b). ADM_{CP} was affected by W treatment ($P < 0.001$) and ratoon cycle ($P < 0.001$) (Table 4). After

5 MAH, ADM$_{CP}$ decreased rapidly due to sugarcane canopy closure except during second ratoon in the rainfed treatment. The higher ADM$_{CP}$ was also observed during the second ratoon in the rainfed treatment with 3.21 t ha^{-1}. This result was related with higher radiation reaching the companion plant (Figure 5c). W treatment and ratoon cycle affected the fraction of incident radiation (fPAR) reaching the companion plant (Table 4). fPAR was lower in the irrigated treatment than in the rainfed treatment ($P < 0.001$). The maximum cumulated fPAR was observed during the second ratoon in the rainfed treatment then the ADM$_{SC}$ and LAI of the sugarcane during growth were the lowest.

Table 4. Analysis of variance (ANOVA) of the random-effects models built to assess the influence of W, N, CS treatments, the age (MAH), and the ratoon cycle on sugarcane and companion plant variables. P values were abbreviated as (.), *, **, and *** when lower than 0.1, 0.05, 0.01, and 0.001, respectively. ns = non-significative. Only significant interactions are shown.

Variables	**W**	**N**	**CS**	**MAH**	**Ratoon cycle**	**2nd order interaction**	**3rd order interaction**
ADM$_{SC}$	(.)	**	**	***	***	CSxCycle ** WxN* ; WxMAH*** ; WxCycle*** NxCycle*** ; MAHxCycle***	WxNxCycle** WxMAHxCycle***
Root_DM$_{SC}$	***	***	*	***	*	WxN*	ns
Height$_{SC}$	*	***	ns	***	***	CSxCycle* WxN* ; WxCycle*** ; NxCycle*** NxMAH** ; MAHxCycle***	ns
Nb_Till$_{SC}$	ns	ns	**	***	***	CSxCycle*** WxCycle** ; MAHxCycle***	ns
N_mass$_{SC}$	ns	*	***	***	***	WxCS* ; WxMAH*** ; WxCycle*** CSxCycle** ; NxCycle*** MAHxCycle***	WxCSxMAH** WxNxMAH** WxNxCycle* WxMAHxCycle***
INN$_{SC}$	ns	ns	***	***	***	WxCS* ; WxMAH*** ; WxCycle*** LxCycle* ; NxMAH* ; NxCycle*** MAHxCycle***	WxCSxMAH** WxNxMAH*** WxNxCycle* WxMAHxCycle***
RS$_{SC}$	***	***	ns	(.)	**	WxN*** ; WxCycle* NxCycle** ; MAHxCycle*	ns
Stalk_P$_{SC}$	***	***	ns	***	***	WxN*** ; WxMAH***; NxCycle***	ns
Yield$_{SC}$	ns	**	**		***	WxN* ; NxCycle***	WxCSxCycle*
Lodging$_{SC}$	***	***	*		***	CSxW* WxN*** ; NxCycle***	ns
ADM$_{CP}$	***	ns		***	***	WxCycle*** ; NxMAH* ; MAHxCycle***	ns
fPAR	***	***		***	***	WxN** ; WxMAH*** ; WxCycle*** NxMAH* ; NxCycle*** ; MAHxCycle***	WxMAHxCycle*** NxMAHxCycle*

ANOVA were performed using a linear mixed-effect model on Box-Cox-transformed traits using the plot as random effect.

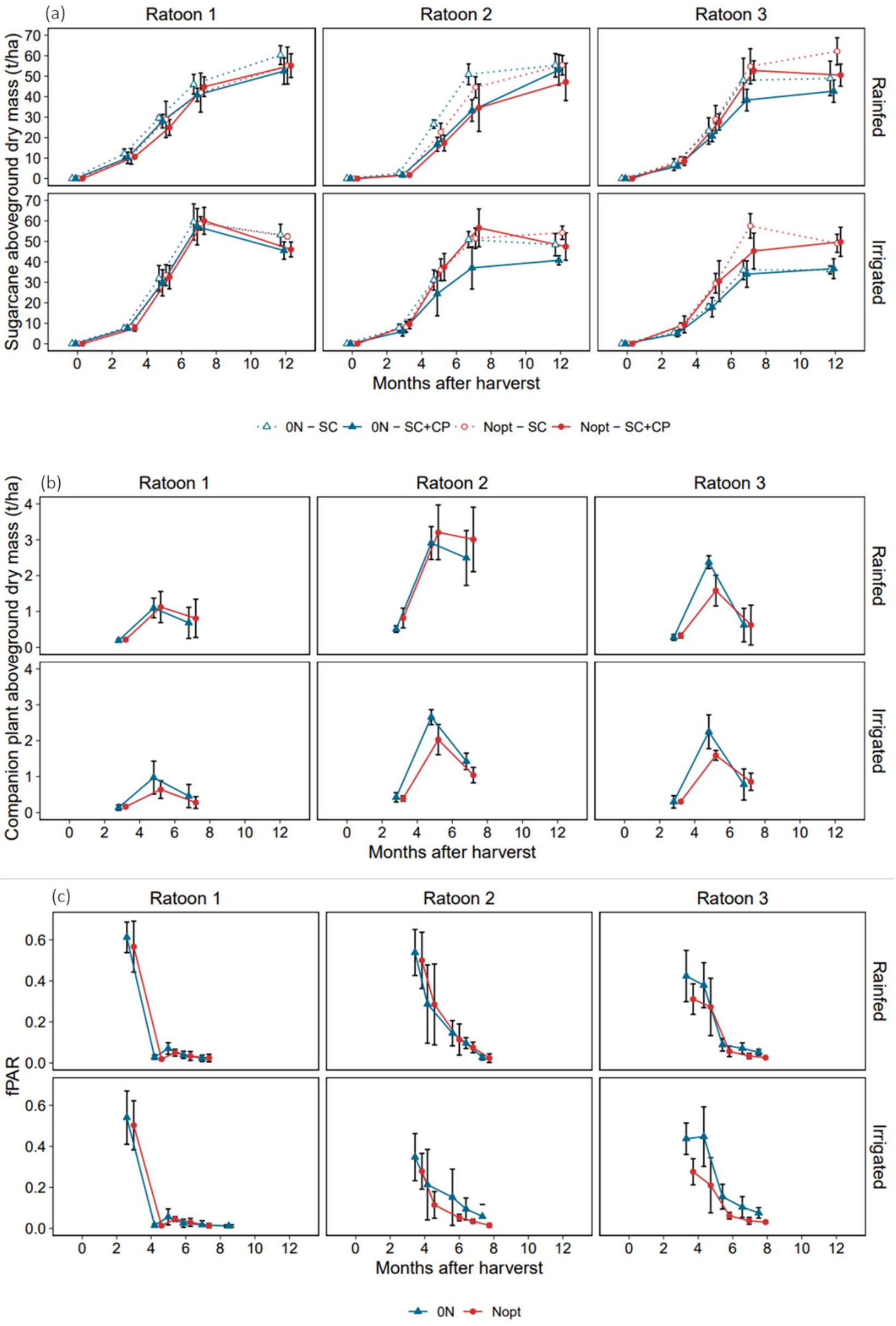

Figure 5. Dynamics of the sugarcane aboveground dry mass (ADM_{SC}) (a), companion plant aboveground dry mass (ADM_{CP}, *C. ensiformis*) (b) and the fraction of incident photosynthetic active radiation above the companion plant (fPAR) (c), in response to W (Rainfed or irrigated), N (0N or Nopt), and CS (SC or SC+CP) treatments, and their interaction for the three ratoon cycles (Ratoon 1: 2019-2020, Ratoon 2: 2020-2021, Ratoon 3: 2021-2022). Dots represent the mean, and bars represent the standard deviation.

3.3. Sugarcane biomass, tiller, and height response to W, N, and CS treatments

The presence of the companion plant reduced sugarcane aboveground biomass (ADM_{SC}), the number of tillers (Nb_Till_{SC}), and root dry biomass ($Root\_DM_{SC}$). ADM_{SC} was influenced by W, N, and CS treatments (Table 4). ADM_{SC} was, on average, 10% lower in the SC+CP treatment than in the SC treatment (Figure 6a). The interaction Ratoon cycle x CS (P = 0.00069) showed that the ADM_{SC} was only affected by the presence of the companion plant for the second ratoon (P = 0.0002). However, the same tendency was observed for the first and third ratoons. ADM_{SC} was, on average, 10% lower in the SC+CP treatment than in the SC treatment. ADM_{SC} was, on average, 36% lower in the combination Rainfed-0N treatment than the Irrigated-Nopt treatment during sugarcane growth.

Tillering was affected by companion plant and W treatment but not by N treatment (Table 4). Nb_Till_{SC} was 8% lower in the intercropped treatment than in the monoculture treatment (Figure 6c, Figure S5). The interaction Ratoon cycle x CS (P = 0.00003) showed that Nb_Till_{SC} was only affected by the presence of the companion plant for the second (-16%) and third (-7%) ratoons. The interaction Ratoon cycle x W (P = 0.007) showed that Nb_Till_{SC} was, on average, 7.6% lower in the rainfed treatment than in the irrigated treatment during the second ratoon.

$Root\_DM_{SC}$ was influenced by W, N, and CS treatments (Table 4). The $Root\_DM_{SC}$ was 12% lower on average in the intercropped treatment than in the monoculture treatment (Figure 6b, Figure S6). $Root\_DM_{SC}$ was also on average 29% higher in the irrigated treatment than the rainfed treatment and 28% lower in the Nopt treatment than the 0N treatment.

Sugarcane height ($Height_{SC}$) were affected by W and N (Table 4, Figure 6d, Figure S7), and the interaction between CS and the ratoon cycle in the variance analysis but not in the pairwise comparison. $Height_{SC}$ was, on average, 11% lower in the combination Rainfed-0N treatment than the Irrigated-Nopt treatment.

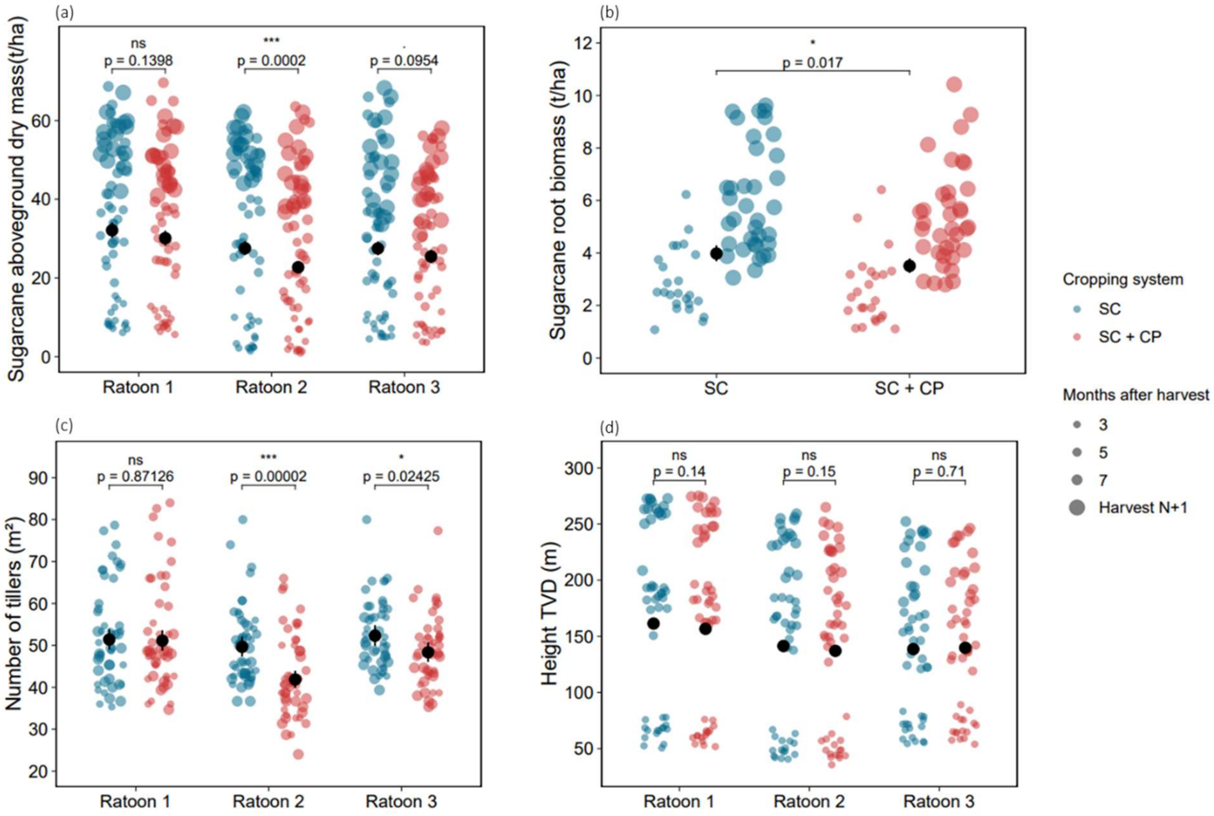

Figure 6. Sugarcane aboveground dry mass (a), number of tillers (c), and height TVD (e) depending on the interaction between the ratoon cycle (Ratoon 1, 2, and 3) and CS treatment (SC vs. SC + CP). Sugarcane root biomass depending on CS treatment (b). The colored dots correspond to the observations, with sizes proportional to the measurement date. The mean values (black point) and confidence intervals (black line) predicted by the linear mixed models with the plot as a random effect are presented. P-values of the pairwise comparisons are indicated.

3.4. Biomass allocation

The presence of the companion plant did not affect sugarcane biomass allocation (Figure 7). The interaction between W and N treatments affected the root-to-shoot ratio and the proportion of stalk on the aboveground dry mass ($P < 0.0001$). The root-to-shoot ratio was 53% higher in Irrigated-0N than in the other treatments. The stalk proportion of the aboveground biomass decreased by 10% for the combination Irrigated-0N than for the others. The individual sugarcane stalk weight was also not affected by the presence of the companion plant (data not shown).

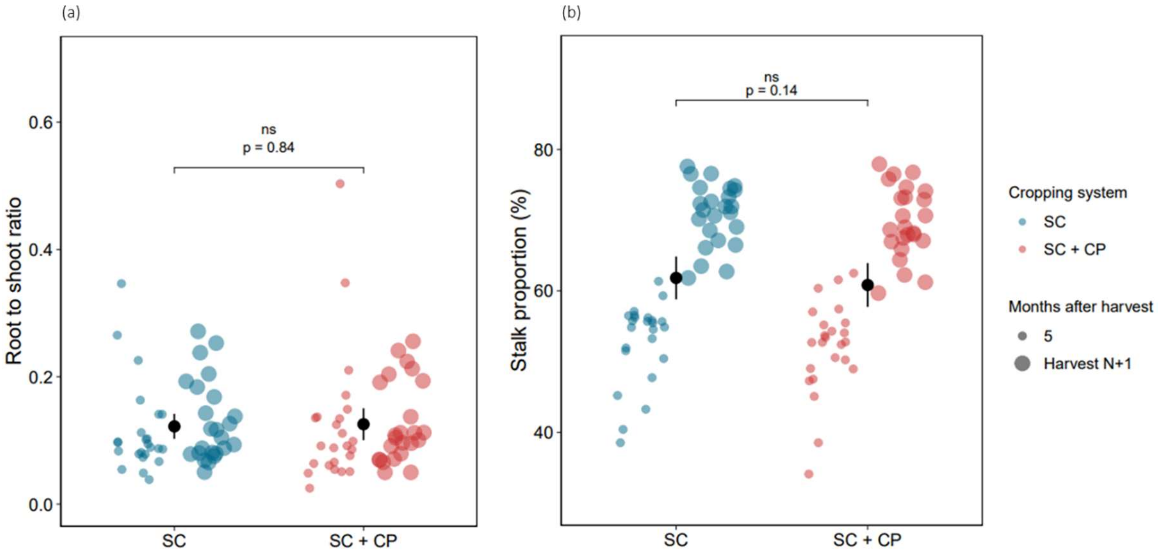

Figure 7. Root to shoot ratio (a) and stalk proportion in the aboveground dry mass (b) depending on the CS treatment (SC vs. SC + CP). The blue and red dots correspond to the observations, with sizes proportional to the date of measurement data values (black point) and confidence intervals (black line) predicted by the linear mixed models with the plot as a random effect are presented. P-values of the pairwise comparisons are indicated.

3.5. Sugarcane yield at harvest and lodging

Sugarcane yield at harvest was influenced by W, N, and CS treatments (Table 4). The maximum yield was observed in Irrigated – sole sugarcane combination of treatment in the first ratoon (Figure 8a). The interaction Ratoon cycle x CS x W ($P = 0.0324$) showed that sugarcane yield was only affected by the presence of the companion plant for the first and second ratoon in the irrigated treatment, with on average a decrease of 15%. In the rainfed treatment, sugarcane yield also tended to be lower in SC+CP than in SC in ratoons 2 and 3. Sugarcane lodging was influenced by all treatments and ratoon cycle (Table 4). Lodging was 32% higher in irrigated than rainfed treatments. The interaction CS x W ($P = 0.0105$) showed that sugarcane lodging was impacted by the presence of companion plant only in the irrigated treatment (Figure 8b), with an increase in lodging by 25% in SC+CP compared to SC.

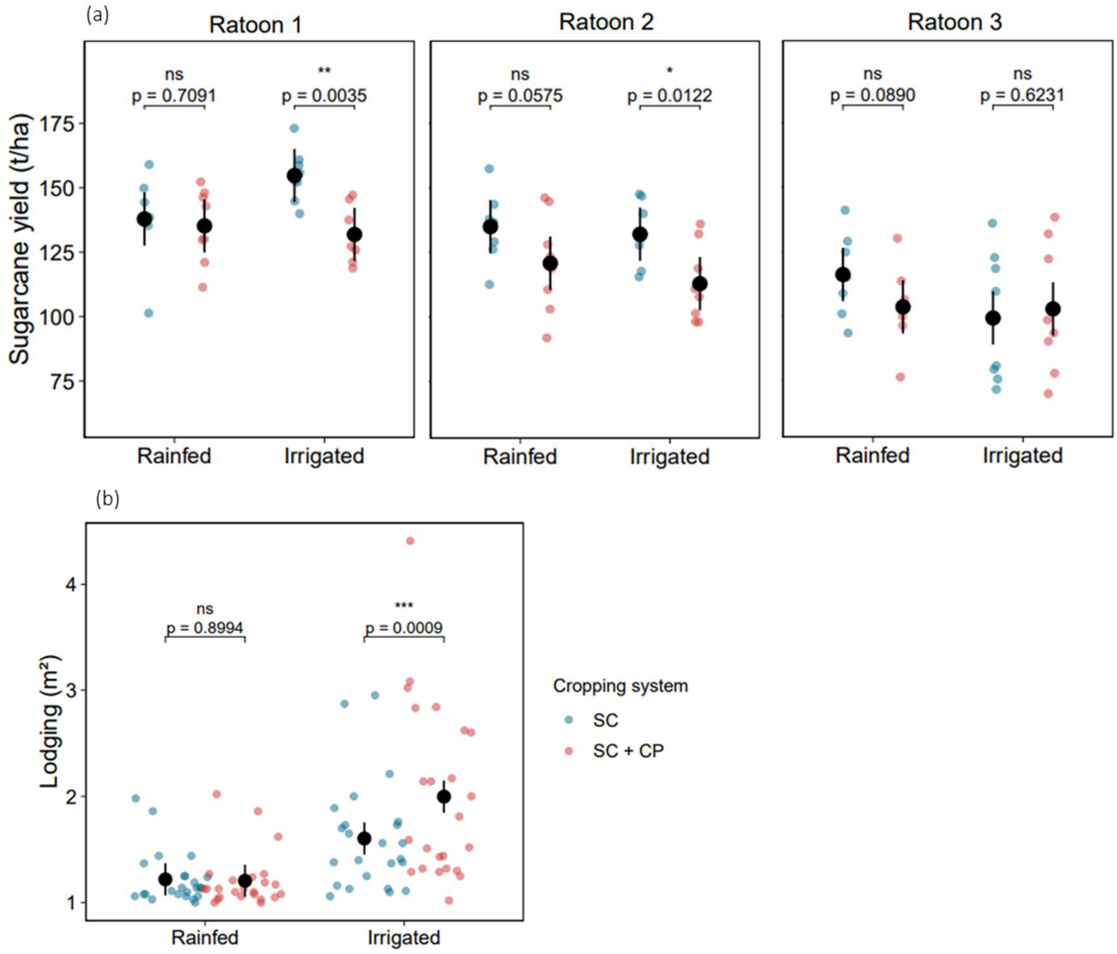

Figure 8. Sugarcane yield (a) depending on the interaction between the ratoon cycle (Ratoon 1, 2, and 3) and CS treatment (SC vs. SC + CP), and lodging (b) depending on the interaction between the W treatment (Rainfed vs. Irrigated) and CS treatment. The colored dots correspond to the observations, with sizes proportional to the measurement date. The mean values (black point) and confidence intervals (black line) predicted by the linear mixed models with the plot as a random effect are presented. P-values of the pairwise comparisons are indicated.

3.6. Structural equation models on sugarcane yield and lodging

A structural equation modeling approach was performed to test the influence of the companion plant aboveground dry biomass (ADM$_{CP}$ at 5 MAH) and sugarcane variables (aboveground dry mass (ADM$_{SC}$) and root biomass (Root_DM$_{SC}$), numbers of tillers (Nb_Till$_{SC}$), height TVD (Height$_{SC}$), stalk (Stalk_P$_{SC}$), and root (Root_P$_{SC}$) proportions) on sugarcane yield (Yield$_{SC}$) and lodging (Lodging$_{SC}$). ADM$_{CP}$ had an indirect effect on Yield$_{SC}$ through Lodging$_{SC}$, the Nb_Till$_{SC}$, and Height$_{SC}$ (Figure 9a, Table S3). At the beginning of the growth, ADM$_{CP}$ influenced ADM$_{SC}$ through a decrease in Height$_{SC}$ (-0.24) and Nb_till$_{SC}$ (-0.34). At the end of the growth Yield$_{SC}$ resulted from a positive effect of Height$_{SC}$ (0.69) and ADM$_{SC}$ (0.28) and a negative effect of Lodging$_{SC}$ (-0.41). Lodging$_{SC}$ was directly influenced by ADM$_{SC}$ (0.34) and Root_DM$_{SC}$ (-0.37) and indirectly affected by Nb_Till$_{SC}$ through ADM$_{SC}$ and Root_DM$_{SC}$ (Figure 9b, Table S3). These fixed factors explained 59% and 48% of Yield$_{SC}$ and Lodging$_{SC}$ variability, respectively. On the contrary, Stalk_P$_{SC}$ and Root_P$_{SC}$ did not influence on Yield$_{SC}$ and Lodging$_{SC}$.

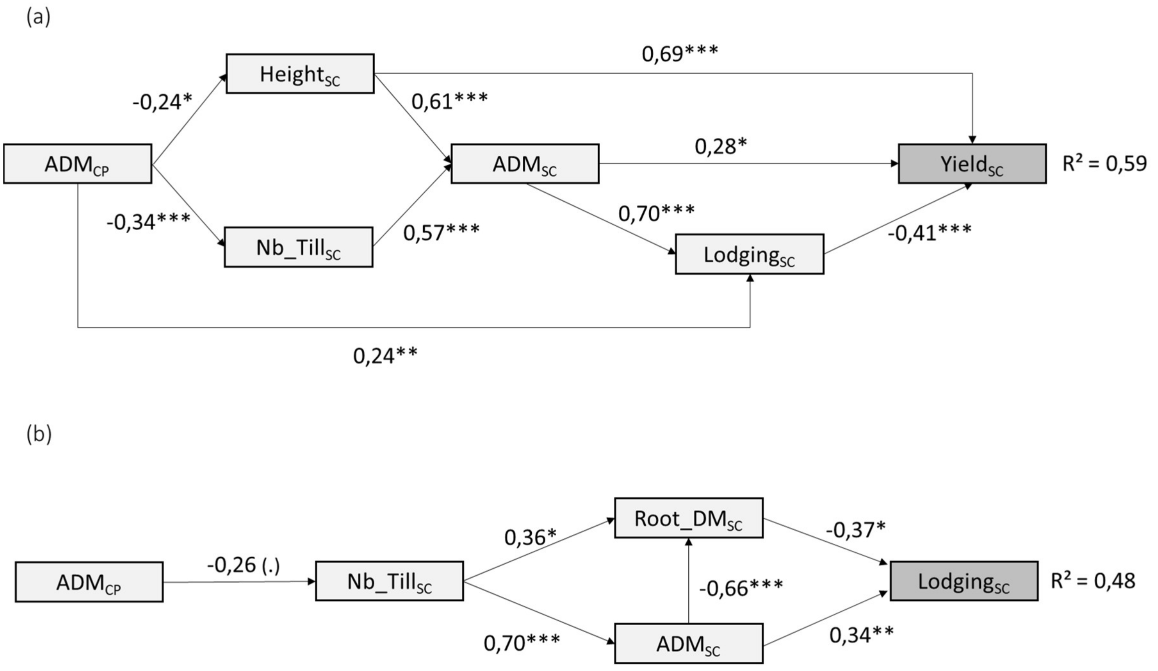

Figure 9. Structural equation model (SEM) showing direct and indirect effects of companion plant biomass and sugarcane parameters on (a) sugarcane yield (Yield$_{SC}$) and (b) lodging (Lodging$_{SC}$). The explanatory variables were the companion plant biomass (ADM$_{CP}$), sugarcane aboveground dry mass (ADM$_{SC}$) and root biomass (Root_DM$_{SC}$), height (Height$_{SC}$), numbers of tillers (Nb_Till$_{SC}$), and lodging (Lodging$_{SC}$ for (a)). All the explanatory variables were measured at 5 MAH during the companion plant's peak biomass, except for lodging (7 MAH). The black arrows represent the result of the analysis. The P values are indicated by ***, **, *, and (.) when lower than 0.001, 0.01, 0.05, and 0.1, respectively. R^2 are given for the predicted variables. The standardized model estimates were given to compare the relative strengths of predictors for continuous variables.

3.7. Competition for soil N and N fertilizer

The sugarcane N mass and N nutrition index (NNI) were influenced by W, N, and CS treatments (Table 4). The interaction W x CS x MAH ($P < 0.0001$) showed that the nutrition of sugarcane was only affected by the presence of the companion plant in the rainfed treatment at 7 MAH (Figure 10). However, the same tendency was observed for the entire growth cycle in the rainfed treatment. On average, the sugarcane N mass and NNI were 19% and 22.8% lower in the SC+CP treatment than in the SC treatment in the rainfed treatment.

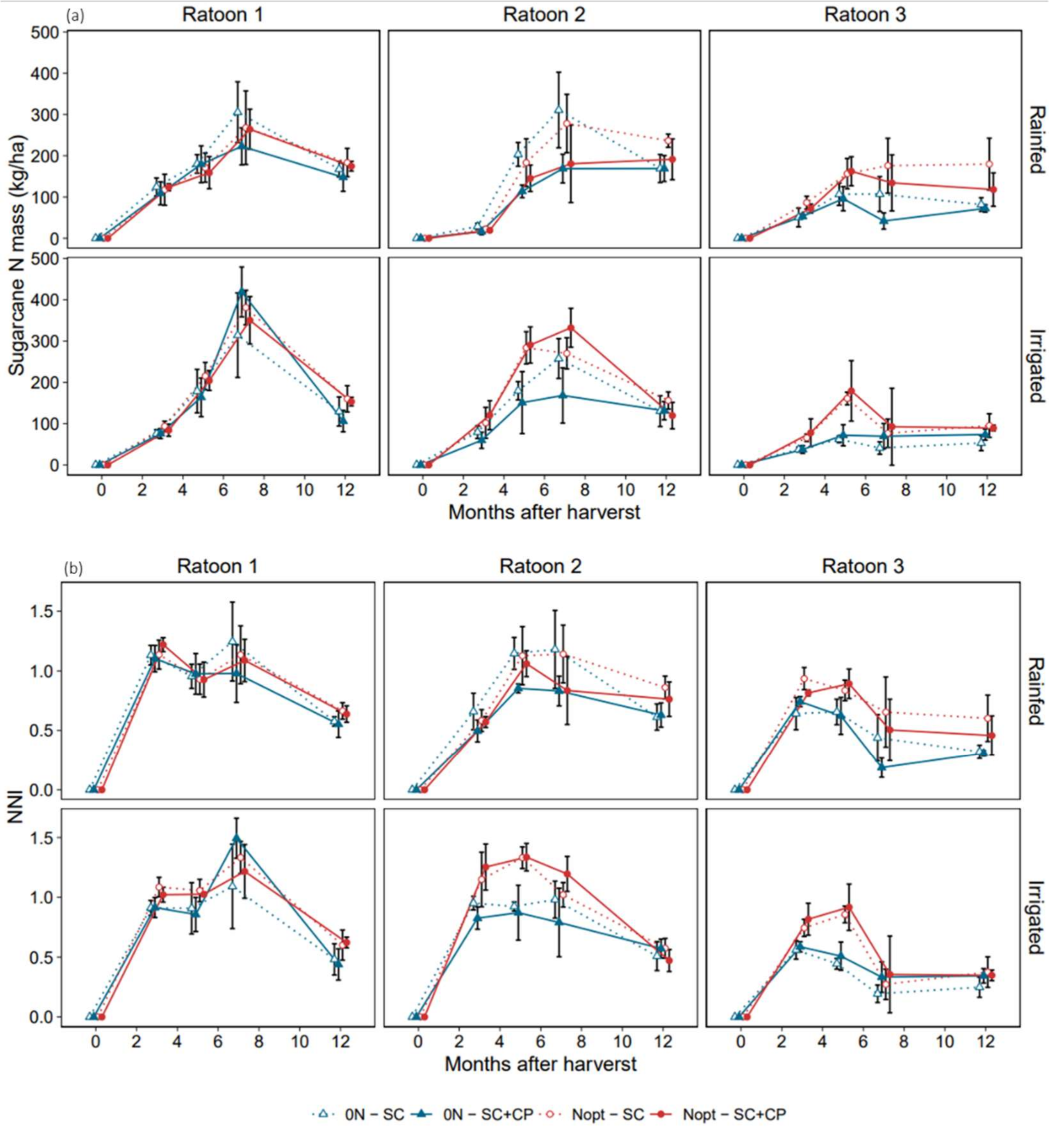

Figure 10. Dynamics of the sugarcane N mass (a), and N Nutrition Index (NNI, b) in response to W (Rainfed or irrigated), N (0N or Nopt), and CS (SC or SC+CP) treatments, and their interaction for the three ratoon cycles (Ratoon 1: 2019-2020, Ratoon 2: 2020-2021, Ratoon 3: 2021-2022). Dots represent the mean, and bars represent the standard deviation.

NdfF and NRE in sugarcane were not affected by the presence of the companion plant for the first and second doses applied ($P < 0.001$) (Figure 11, Table S4, S5). Over its growth cycle, the companion plant took up, on average, 4.5% of its N demand from ^{15}N labeled fertilizer (Table S4, S5). For the sugarcane, there was a maximum NdfF of 30.6 % 3 months after harvest of the first dose applied, with an average NdfF of 8.33 % over the growth cycle and the two application dates

(Table S4, S5). Regarding the total amounts applied, sugarcane recovered, on average, 10.1% of the ^{15}N labeled fertilizer at harvest (Figure 8b). The companion plant used a negligible part of the N fertilizer with 1.5% and 2.3% of the first and second doses. The total fertilizer recovery was similar among the treatments, with an average of 7.9% over the growth cycle for the two application dates. Throughout the experiment, fertilizer recovery in mulch and the different soil depth layers showed a similar distribution among treatments and date of application. Most fertilizers were recovered in the top 0-5 cm. At harvest, 19.5% of the fertilizer applied was recovered in topsoil (0-5 cm). Only minor amounts of the fertilizer were transferred to the deeper soil layers.

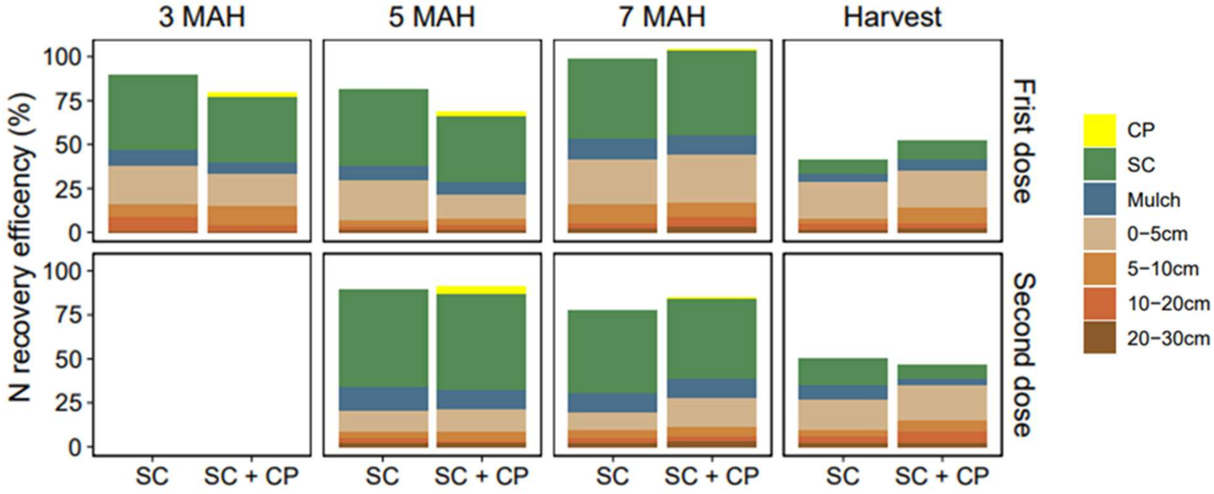

Figure 11. N recovery efficiency of the fertilizer in the companion plant aboveground biomass, sugarcane aboveground biomass, mulch, and soil (at 0-5cm; 5-10cm; 10-20cm; 20-30cm depth) as affected by the time of N application (first and second dose) and CS treatment (SC vs. SC + CP). The first dose of ^{15}N urea was applied at 1 MAH and the second at 3 MAH during ratoon 1.

3.8. Soil water content

The soil water content (SWC) dynamic was similar among the different treatments during Ratoon 1 (Figure 12), with a decrease of SWC at the end of the growth cycle during the dry season. This effect was particularly pronounced for rainfed treatment. There were two periods with a decline of the SWC at about 90 and 180 days after harvest in the rainfed treatment, corresponding to a period of low precipitations. The presence of the companion plant did not affect the SWC for the entire soil profile, indicating that competition for water was low during the first ratoon. This result was consistent with the non-significant interaction between W and SC during the first ratoon on sugarcane growth. However, W treatment and its interaction with SC treatment affected the sugarcane growth during the second and third ratoon, which might be resulted from soil water competition. Nonetheless, SWC measurements were not available in the second and third ratoon.

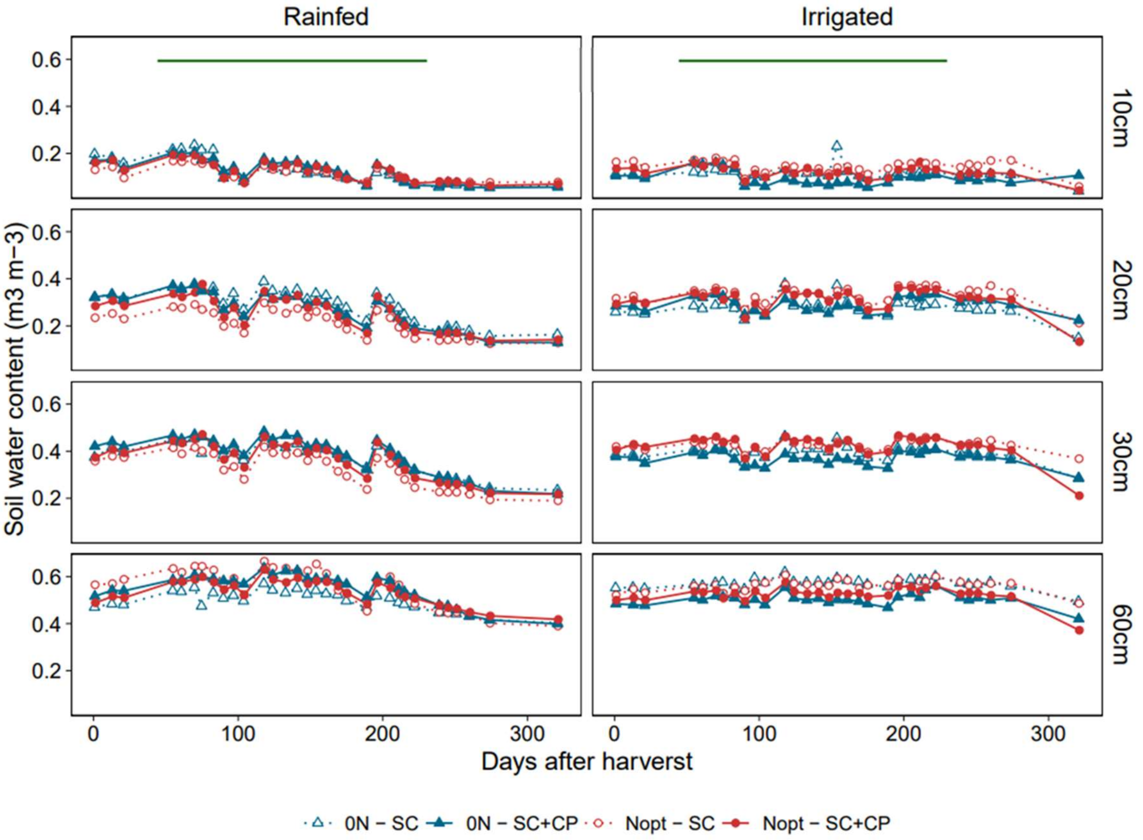

Figure 12. Soil water content (m m^{-3}) between SC and SC + CP treatment (b) during ratoon 1 (2019-2020) as affected by W treatment and soil depth (10cm; 20cm; 30cm; 60cm soil depth). The green arrow represents the cohabitation period of the sugarcane and the companion plant. The SWC were not measured during the second and third ratoons.

4. Discussion

4.1. Sugarcane growth and biomass allocation in intercropping

Sugarcane growth and yield were generally affected by the presence of the legume companion plant supporting evidence of interspecific competition. On average, the decrease in sugarcane yield was about 9% in the intercropped treatments compared to the monoculture treatments, where it was about 3.34% worldwide in our meta-analysis on sugarcane-legume intercropping systems (Viaud et al., 2023).

The negative impact of intercropping on sugarcane growth was proportional to the development of the companion plants in the inter-row, as seen in a previous meta-analysis (Viaud et al., 2023). The sugarcane growth was particularly affected when the companion plant grew vigorously during the second ratoon in the rainfed treatment suggesting that sugarcane is sensitive to competition from legumes. Conversely, several studies have shown that legume species are highly sensitive to light competition, compared to below-ground competition for nutrients (Mantino et al., 2021; Xiao et al., 2004). High legume biomass in our study was achieved when sugarcane initial growth was limited by rainfall availability (second ratoon), delaying the canopy closure with higher radiation reaching the companion plant. Consequently, as seen in other intercropping systems the initial growth of the main crop will be determinant in terms of competitive advantage during the crop cycle (Yu et al., 2016).

Unlike other crops, sugarcane yield is directly related to vegetative growth, as stalks are the main components of yield. We hypothesize that a decrease in yield might be related to a shift in biomass allocation to stalk, leaves, and roots because above-to-belowground ratio of sugarcane is known to be highly variable depending on environmental conditions (Chevalier et al., 2023). However, we did not find change in biomass allocation due to the presence of the companion plant contrary to other cultures with greater plasticity (such as wheat: Ajal et al., 2022 or maize: Yang et al., 2022). Therefore, the decrease in yield resulted from a decrease in sugarcane growth.

Similarly, to above-ground biomass, the root biomass was reduced by 12% in the SC+CP treatment compared to the monoculture treatment, which, with the addition of the co-occurrence of legume roots, could induce a decrease in sugarcane root colonization and access to soil resources (Christina et al., 2023). The impact of CP on sugarcane roots were still present at harvest, 4 months after the end of the co-growth stage. This result supports a low plasticity of the sugarcane, which is disadvantageous as the phenotypic plasticity of roots is an important process in the recovery growth of intercropping system (Liu et al., 2020). These results were consistent with a previous study conducted in a planting year on Reunion Island, where sugarcane showed a decrease in root density and root avoidance strategy when grown with *C. ensiformis* (Christina et al., 2023). Research is scarce on the impact of intercropping on sugarcane root systems, and the few previous studies showed contrasted impact of intercropping depending on species and crop cycle (Lovera et al., 2021). The decrease in the quantity of roots observed in our study could lead to indirect competition through a decrease in the volume of soil explored by the roots and therefore of the available soil resources.

4.2. Early and late response of sugarcane traits to the presence of legume

Our study shows that the growth of sugarcane is affected by the presence of the companion plant at the beginning of the crop cycle through tillering and at the end of the growth through lodging.

At the beginning of the growth, the presence of the companion plant had a negative impact on the number of tillers but did not affect the height and single cane weight. Compared with sole sugarcane, the number of tillers was reduced by 8% in intercropping. Previous studies have also documented that sugarcane tillering was reduced by intercropping (Rasool et al., 2011; Rehman et al., 2014; Yang et al., 2013). The authors attributed this diminution to a decrease in light interception and soil temperature when the companion plant fully covered the space in the sugarcane inter-row. However, this interpretation can't be fully applied to our case as the number of tillers was already decreased by the presence of legumes at 3 MAH while it covered only 15 to 30% of the inter-row at this period, depending on the years and treatments. Thus, this diminution might be more related to soil resource availability (water and nitrogen), as observed in previous studies on monocropping (Gomathi et al., 2015; Zhao et al., 2014).

At the end of the growth, the aboveground biomass stagnated or decreased between 7 MAH and harvest in the irrigated treatment, as a result of sugarcane lodging. Lodging can cause the plant to stop growing or break in the most severe forms (Li et al., 2019) and reduces the quality of mechanical harvesting, leading to losses at harvest time (Yang et al., 2020). Unexpectedly, the presence of legume increased sugarcane lodging all three years. Such impact has never been observed in the literature, but lodging is still a poorly studied process. Lodging is known to be related to wind, stem weight, and water saturation in soil (Li et al., 2019; Park et al., 2005; Singh et al., 2002), which can explain why we observed lodging only in the irrigated treatment. Nonetheless, previous studies also suggested a relation between lodging and root development. As an example, Yang et al. (2020) have shown a relation between the amount of root in surface horizons and lodging resistance. Other studies have shown that lodging is accentuated for varieties with low roots (Jongrungklang et al., 2018). Our results highlighting a correlation between root biomass and lodging agreed with these previous studies. Consequently, the decrease in root biomass due to legume presence observed from 5 months onwards could explain the higher lodging. The lodging response to intercropping has not been observed in previous experiments in Reunion Island (see list in Chapter 1), but it was never actually measured. One explanation is that in our trial the sugarcane has particularly little root quantity compared to other experimental trials on the island (Chevalier et al., 2023). Nonetheless, lodging should be considered in future intercropping studies, as Reunion Island is subject to frequent cyclones (Christina et al., 2021a), and extreme events with heavy rainfall and strong winds are becoming increasingly frequent worldwide (Field et al., 2012).

4.3. The role of water and nitrogen on interspecific interactions

In this study, we explored the effects of nitrogen and water availability (fertilization, irrigation or annual variability) on sugarcane response to legume intercropping.

NdfF and NRE in the sugarcane were unaffected by the companion plant, showing that there is no competition for the urea fertilizer. To our knowledge, the competition for urea fertilizer has never been measured in intercropping systems with legumes. In our system, such an absence of competition was expected due to the management. Indeed, in our sugarcane cropping system, fertilizer is applied on the sugarcane row before legume sowing (first application) or only one month after sowing (second application). Additionally, cereal intercropping meta-analyses suggested that fertilization could give a competitive advantage to the main crop (Xu et al., 2020) even if the response is highly variable across studies (Pelzer et al., 2014). Our results did not reveal any competitive advantage of fertilization (no N x CS interaction), as reported in other sugarcane intercropping studies (He et al., 2018; Nadeem et al., 2020; Teshome et al., 2014; Tian et al., 2020).

Contrary to N fertilization, our results suggest a higher impact of legume on sugarcane growth when soil N is limited. In the rainfed treatment in this study, in years with dry periods (years 2 and 3), the sugarcane NNI decreased by 23% in the presence of the companion plant, in contrast to the irrigated treatment, where soil mineralization is potentially greater. Previous meta-analyses also showed that competition was greater in poor soils than in rich ones in sugarcane (Viaud et al., 2023) or cereal legume intercropping systems (Xu et al., 2020). Nevertheless, the amount of N in the legume coming from the soil (4-31 kgN ha^{-1}, see Chapter 3) is relatively low compared with the decrease in N in the sugarcane (50-100 kg ha^{-1}) due to legume presence even if it could have an impact depending on the coexistence period of legume and sugarcane demand. Such observation suggests that other process than a decrease in soil mineral N (N uptake by the legumes) could occur. The higher N stress index of sugarcane in presence of legume when soil N is limited could also result from a decrease in root colonization by sugarcane. Indeed, previous studies in sugarcane plant crop have shown a decrease in soil volume accessible by sugarcane by 27% in presence of legume (Christina et al., 2023).

Similarly to Viaud et al. (2023), our result did not show any interaction between irrigation and the sugarcane growth response (at the beginning of the crop cycle). Nonetheless, in years with dry periods at the beginning of the growth (like the 2nd year in our study), the sugarcane growth was delaying, making it less competitive with the legume (greater impact on biomass the 2nd year). Similarly, Viaud et al. (2023) showed a tendency for low rainfall without irrigation to have a greater impact on yield, which has also been observed in other systems (Daellenbach et al., 2005; Roy et al., 2015; Waddington et al., 2007). Irrigation at the beginning of the crop cycle to stimulate sugarcane growth therefore seems to be a key factor in intercropping systems.

5. Conclusion

Our study suggests that sugarcane has a low plasticity regarding biomass allocation in response to the presence of legumes. The presence of a legume reduces yield by 9% on average through an overall decrease in both above-ground and below-ground biomass. At the start of growth, the decrease in biomass is linked to a reduction in the number of tillers, adding the effect of lodging amplified by the presence of legumes at the end of growth. Despite the absence of Nitrogen fertilization uptake by legumes, the fertilization does not give sugarcane a competitive advantage. However, irrigation ensures better sugarcane growth at the beginning of the cycle, making it more competitive with the legume, although increasing the risk of lodging at the end of the growing year. Our results suggest that the response of the sugarcane root compartment in the presence of legumes is a crucial factor explaining yield response. Future studies should investigate these interactions in contrasting soil types and varieties having different above-ground and below-ground growth strategies.

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Chapter 3: Sources and fate of Nitrogen from legumes in sugarcane intercropping system.

Ce travail a été réalisé sur un essai expérimental de l'unité AIDA sur la station expérimentale de La Mare à La Réunion. Les personnes impliquées ainsi que leurs contributions respectives à ce chapitre de thèse sont listées ci-dessous.

Pauline Viaud a effectué le suivi de l'essai, la gestion des échantillons, l'analyse des données et la rédaction du chapitre. **Antoine Versini** a apporté la méthodologie pour la modélisation des flux avec Hydrus et le suivi de l'azote ^{15}N dans les microplots, à l'analyse des données et à la rédaction du chapitre. **Mathias Christina** a participé au suivi de l'essai, à l'analyse des données et à la rédaction du chapitre. **Krishna Naudin** a participé à l'interprétation des résultats et à la rédaction du chapitre. L'équipe technique de eRcane a également apporter son appui lors des semis des légumineuses. **Christian Hossann** a réalisé les analyses d'azote ^{15}N au laboratoire PTEF de l'INRAe de Nancy.

1. Introduction

Nitrogen (N) is an essential nutrient but often a limiting factor in agricultural systems. Current agricultural systems increasingly rely on N fertilization to meet the growing demands of an expanding world population. However, excessive and inefficient use of mineral N fertilizers can have harmful consequences for the environment (Sutton et al., 2013). In this context, introducing legumes in cropping systems is a promising way to reduce dependence on N fertilizers thanks to their ability to fix atmospheric N_2 (Zhao et al., 2022). It is generally accepted that legumes can make substantial net N contributions to soil fertility. In sugarcane systems, several studies have demonstrated the positive effect of legumes as a rotation break crop. For example, Shoko et al. (2007) reported that fallow soybeans could substitute basal fertilizer N application without decreasing sugarcane yield at planting. However, rotation only occurs every 7 to 15 years in sugarcane crops (varying from country to country), which limits the benefits of rotation on soil fertility.

Sugarcane crops are well-suited for intercropping due to their wide row spacing and slow growth in the initial stage. Intercropping has become increasingly popular in sugarcane planting in recent years, as it can improve crop productivity and economic income and help control pests such as diseases, insects, and weeds (Berry et al., 2009; Nazir et al., 2002). However, the impact of legumes on soil fertility and sugarcane nutrition in intercropping systems has still been poorly investigated in the literature.

The biomass and N fixed by legumes in sugarcane intercropping systems are much lower than those of legumes in a rotation. (e.g., from 13 to 60 kg N ha^{-1} fixed in an intercropping system (de Resende et al., 2003; Tian et al., 2020) vs. from 90 to 246 kg N ha^{-1} fixed in a rotation system (Shoko et al., 2007; Tian et al., 2020)). Intercropping can lead to changes in biological nitrogen fixation (BNF). It is generally observed that the contribution of N_2 fixation to total N accumulation in legumes is higher when intercropped with cereal than in sole crops due to the strong competitiveness of cereals for N (Corre-Hellou et al., 2006; Hauggaard-Nielsen et al., 2009; Jensen, 1996). Nevertheless, the amount of N_2 fixed is also influenced by plant growth, which can be significantly hindered in intercropping due to the competition for light, as discussed in Chapter 2.

In intercropping systems, legume residues are generally left on the soil and mixed with sugarcane residues rather than buried as in rotation systems. The N mineralization occurring during decomposition is commonly slowed when residues are not incorporated into the soil (Jahanzad et al., 2016). In contrast, the mixture of legume (low C: N ratio) and sugarcane residues (high C: N ratio) could lead to accelerated decomposition by the non-additive or synergistic effect (Liu et al., 2020).This trend could be explained, among other mechanisms, by the net transfer of N from poor-N components to rich-N components during the mulch decomposition (Schimel and Hättenschwiler, 2007; Versini et al., 2016). A variable part of the legume N could, therefore, be immobilized in the sugarcane mulch, limiting the transfer of N to the sugarcane in favor of the soil compartment.

Legumes are expected to feed the soil organic matter pool that drives the sugarcane system's N fertility (Dourado-Neto et al., 2010; Stevens et al., 2005). Overall, the effect of cover crops on soil organic matter varies greatly from one study to another and remains controversial (Chaplot and Smith, n.d.; Poeplau and Don, 2015). Nonetheless, the only study dealing with the long-term effect of legumes on soil properties in sugarcane-legume intercropping systems did not show an increase in soil organic carbon content after 10 years of intercropping (in China, Wang et al. (2020)). This finding could be linked a soil organic matter content that is already high due to the high biomass production of sugarcane and/or to significant losses of legume-N. Indeed, a growing number of studies point out legume-N losses through ammonia volatilization (Glasener and Palm, 1995; Nett et al., 2016), N_2O emissions (Peyrard et al., 2016), and nitrate lixiviation (Quemada et al., 2013).

Although short-term experiments have shown higher available NPK in the soil after legume death (Lian et al., 2019; Solanki et al., 2019), the monitoring of N legume in the soil and plant compartments is hampered by the dilution effect. Nitrogen inputs from legumes are too low to allow a solid assessment of their contribution to soil organic matter buildup or crop nutrition. These contributions can nevertheless be studied from long-term trials or thanks to isotopic tracing. Enrichment in isotope ^{15}N during legume growth can be used to trace the fate of residual nitrogen in the soil-plant system (Versini et al., 2014; Zeller et al., 2001) using a protocol adapted to sugarcane (Poultney et al., 2020).

In the present study, we investigate the sources and fate of legume-N to verify the added value of the sugarcane x legume intercropping system compared to the conventional system regarding soil N fertility. The specific objectives of this study were (i) to quantify the N_2 supplied to the cropping system by biological N fixation, (ii) to investigate the fate of legume-N along the decomposition continuum from residue to soil organic matter, and (iii) to assess the legume-N contribution to sugarcane nutrition as well as potential losses to the environment.

For this purpose, we chose a study case: sugarcane x *Canavalia ensiformis* (jack bean) intercropping, based on previous studies in Reunion Island (Christina et al., 2021; Mansuy et al., 2019). The amount of N_2 fixed by the legume was measured during three intercropping cycles. Decomposition and N release of *C. ensiformis* residues and sugarcane mulch were assessed using a litterbag approach. The fate of N from legumes in the soil-plant system was studied using ^{15}N-labeled legumes. The soil moisture and mineral N were monitored to estimate the N fluxes based on a modeling approach throughout the soil profile.

2. Methods

2.1. Study site and experimental design

Experiments were carried out on the SALSA trial described in Chapter 2 (see sections 2.1 and 2.2 in the Methods section). The trial consisted of three-year ratoon sugarcane with three crossed treatments: irrigation (W) or rainfed (0W), nitrogen fertilization (Nopt) or no fertilization (0N), and sugarcane x companion plant intercropping (SC + CP) or sugarcane monocropping (SC), resulting in eight modalities (W+Nopt+SC+CP, W+Nopt+SC, 0W+Nopt+SC+CP, 0W+Nopt+SC, W+0N+SC+CP, W+0N+SC, 0W+0N+SC+CP, 0W+0N+SC). Companion plant biological fixation and N mass (section 2.2) will be presented for all treatments. The other measurements were performed only in the irrigated and fertilized treatments (W+Nopt+SC and W+Nopt+SC+CP).

2.2. Legume biological N fixation and N mass

The aboveground and root biomass of the companion plant (*Canavalia ensiformis*, jack bean) were measured at the flowering stage over three cropping seasons (March in ratoon 1 - 2020, ratoon 2 - 2021, and ratoon 3 - 2022). The fresh aboveground biomass was estimated from 2 samples of 1.5m^2 in each plot. A sub-sample was oven-dried at 60°C for 72h and weighed to obtain dry matter content. The root biomass was measured in 2021 and 2022 with a soil core of 20 cm diameter and 30 cm depth centered on one individual in each plot. The aerial biomass of this individual was measured to calculate the root-to-shoot ratio. Soil samples were washed using a 0.5 mm sieve to extract legumes roots. The roots of sugarcane and legume were distinguished through color (brown for sugarcane and white for *C. ensformis*) and ramification (highly ramified for sugarcane and poorly ramified for *C. ensiformis*). The roots were dried at 60°C for 72 hours to obtain root dry mass. Aboveground and root sub-samples were taken to measure the C and N content (see chemical analysis section 2.6). The root-to-shoot ratio and the root N content measured in 2021 and 2022 were used to estimate the root biomass in the 0-30cm layer and root N content in 2020.

The proportion of N derived from the atmosphere (Ndfa) in the aboveground mass of *C. ensiformis* was assessed using the ^{15}N natural abundance method (Shearer and Kohl, 1986, see chemical analysis section 2.6):

$$Ndfa\ (\%) = \left[\frac{\delta^{15}N_{ref} - \delta^{15}N_{C.ens}}{\delta^{15}N_{ref} - \beta}\right] * 100 \qquad (1)$$

$$Ndfa\ (kg\ N\ ha^{-1}) = \frac{Aboveground\ N\ mass * \%Ndfa}{100} \qquad (2)$$

Where $\delta^{15}N_{ref}$ is the $\delta^{15}N$ of a non-fixing reference plant, $\delta^{15}N_{C.ens}$ is the $\delta^{15}N$ of *C. ensiformis*, β is the $\delta^{15}N$ of *C. ensiformis* relying on atmospheric N_2 as a sole source of N.

Guizotia abyssinica was used as reference plant during ratoons 1 and 2. Non-legume weeds were used during ratoon 3 because the reference plants did not germinate. During the first ratoon, the reference plants were growing in the buffer zone of the trial (see Chapter 2, Section 2.2, Figure 1). In subsequent years, reference plants were cultivated near the sampling area, ensuring that the

$\delta^{15}N$ values of the reference plants closely matched the $\delta^{15}N$ values of the soil N utilized by the legume, as recommended by Unkovich et al. (2008).

As β value, we used the lowest detected $\delta^{15}N$ in the field, resulting in a β value of −1.6‰ (Motisi et al., 2007; Oberson et al., 2013). Our β value was lower than the β value used earlier for *C. ensiformis* (i.e., -1.1‰ in Becker and Johnson, 1998).

2.3. Litter bags

2.3.1. Experimental setup

Decomposition and N release of *C. ensiformis* residues and sugarcane mulch (i.e., dead leaves residues left after harvest) were assessed using a litterbag approach. This experiment was conducted during the first ratoon (2019-2020) with 5 residue mixtures as treatments. The plant materials were collected during the harvest of the plantation in October 2019 of the SALSA trial. The materials were stored at 5°C from the harvest until the litter-bags deposition on the field in November 2019. The litter treatments were:

- 100% legume companion plant (100CP),
- 100% legumes plus 100% sugarcane mulch (100CP/100SC),
- 100% legumes plus 50% sugarcane mulch (100CP/50SC),
- 50% legumes plus 100% sugarcane mulch (50CP/100SC),
- 100% sugarcane mulch (100SC).

The dry mass of the 100SC and 100CP both corresponded to 8 t ha^{-1}, based on the average sugarcane mulch at harvest (7.77 t.ha^{-1}) and companion plant (8.31 t ha^{-1}) biomass measured during the plant crop. For the companion plant, the proportion of stalks, leaves, and pods measured at legumes' maturity during the plantation was used (Table 1). The N mass of the 100SC and 100CP corresponded to 40 kg N ha^{-1} and 250 kg N ha^{-1}, respectively.

Table 1. Initial N concentration, C: N ratio of the residues, and mass proportion of the legume compartments.

Residues		N concentration (%)	C:N ratio	Mass proportion (%)
Sugarcane mulch		0,50	88 :1	-
Canavalia ensiformis	Stalks	1,45	31 :1	32
	Leaves	3,98	11 :1	64
	Pods	3,60	12 :1	4
	Aboveground	3,13	14 :1	-

2.3.2. Field procedures

Litterbags were prepared using a white polyvinylchloride garden grid with a mesh of 1 cm to avoid macrofauna exclusion, with 30 x 30 x 15 cm internal dimensions. A 1 mm nylon mesh was added at the bottom of litterbags, allowing the retention of residues during fragmentation. Each treatment was replicated 12 times. The litterbags were randomly disposed of in the inter-row of one plot of the trial in the W+Nopt+SC+CP treatment. The soil areas beneath the litterbags were free of any visible particulate organic matter before litterbag deposition. The mulch was uniformly arranged around the litterbags to ensure continuity for macrofauna. The litterbags were fixed to the soil surface using U-shaped metallic clamps.

2.3.3. Sampling and chemical analyses

Three litterbags per treatment were sampled at 1, 3, 5, and 7 months after on-field deposition to determine the biomass and N content of the remaining mulch. Residue components were separated by hand between sugarcane mulch, legume residue, and a residual mixture too decomposed to be identified. Samples were oven-dried at 60 °C for 72h and weighed to obtain dry matter. Sub-samples were taken to measure the C and N content (see section 2.6). The proportion of sugarcane and legumes in the residual mixture was determined using ^{13}C isotopic abundance (see section 2.6).

2.4. ^{15}N labeled legumes fate.

The fate of N from legumes in the soil-plant system was studied using ^{15}N-labeled legumes. This experiment was repeated twice (2020-2022 and 2021-2022) in the W+Nopt+SC+CP treatment.

2.4.1. ^{15}N legume labelling

C. ensiformis were grown in inert river sand and gravel substrate in a greenhouse and supplied with a nutrient solution containing $K^{15}NO_3$ (10 atom% ^{15}N excess) until plant maturity (4 months after sowing). Irrigation was applied to avoid any stresses through growth. The final isotopic enrichment of the aboveground residues was 0.734 and 2.017 atom% ^{15}N for the first and second years of experiments.

2.4.2. Microplots for field ^{15}N labelling experiment.

The labeled legumes were harvested and placed in microplots (3 replicates) each year at the beginning of June (corresponding to the period when legumes naturally die in our experiments). The microplots of 3 m^2 (2 m x 1.5 m) were installed in the treatment W+Nopt+SC+CP based on Trivelin et al. (1994). PVC rings (with a diameter of 20 cm and height of 10 cm) were set up next to the microplots for soil and mulch sampling to minimize the variability of ^{15}N abundance measurements due to mulch and soil heterogeneity. The legume residues were placed above the sugarcane mulch after removing the non-labeled legumes presented in the field. The amount of sugarcane mulch was calculated by averaging the measurements taken from four 3 m^2 samples within the same treatment when the microplots were established. The dry mass of sugarcane mulch and labeled legume residues corresponded to 7 and 4 t ha^{-1} in the first year and 5 and 3 t ha^{-1} in the second year. The N mass of sugarcane mulch and labeled legume residues corresponded to 23 and 47 kg N ha^{-1} in the first year and 29 and 91 kg N ha$^{-1,}$ respectively, in the second year.

2.4.3. Soil, mulch, and plant sampling

The ^{15}N signature of sugarcane was measured at 6 dates for the first experiment and 4 dates for the second (Table 2). The ^{15}N signature of mulch and soil was measured at 2 dates for the two experiments (Table 2).

Table 2. Sampling dates for the ^{15}N signature of sugarcane, mulch, and soil for the first and the second experiments, and ammonium and nitrate concentrations during the second experiments ("x" corresponding to one sampling date).

	Ratoon 1			Ratoon 2						Ratoon 3	
	June 2020	July 2020	Oct. 2020	March 2021	June 2021	July 2021	August 2021	Sept. 2023	Oct. 2021	March 2022	Oct. 2022
Application of ^{15}N labeled residues	①				②						
Sugarcane		①	①	①		②			① ②	① ②	① ②
Mulch + Soil		①	①			②			②		
Mineral N soil					x x x	x x	x	x	x		

After collecting the mulch from one PVC ring, the soil was sampled at four different depths: 0-5, 5-10, 10-30, and 30-50 cm. The 0-5 and 5-10 cm soil layers were excavated, and the 10-30 and 30-50 cm soil layers were sampled with a manual auger.

The leaf+1 (first leaf below the terminal visible dewlap) represented ^{15}N abundance in the sugarcane plant (Poultney et al., 2020). Leaves+1 were collected and pooled from two central sugarcane stalks of each microplot. As the neighboring plants absorb ^{15}N applied to the microplot (Trivelin et al., 1994), two leaves+1 were collected and pooled from the adjacent rows beside the microplot. Three individual sugarcane shoots were collected at the center of each microplot at harvest to verify the reliability of leaf+1 to represent the ^{15}N abundance in the sugarcane plant.

For each compartment (i.e., mulch, soil, and sugarcane), an unlabeled control was sampled with the same protocols to determine the natural abundance at each sampling date.

2.4.4 Nitrogen derived from legume residues.

The proportion of N derived from the legume residues (NdfCP) of the considered compartment was determined by the following formula:

$$NdfCP = \left[\frac{a-b}{c-d}\right] * 100 \qquad (1)$$

Where a is the abundance of ^{15}N atoms of the considered compartment (i.e., soil, mulch, sugarcane,) (%), b is the natural abundance of ^{15}N atoms of the unlabeled control sample (%), c is the abundance of ^{15}N atoms% of the legume residue (%), and d is the natural abundance of ^{15}N atoms of air (i.e., 0.3663 atom%).

The NdfCP of sugarcane and companion plant was calculated as follow :

$$NdfCP_T = NdfCP_M + 2 * NdfCP_A \qquad (2)$$

Where $NdfCP_T$ is the sum of the NdfCP determined from the center of the microplot ($NdfCP_M$) and the adjacent plants beside the microplot ($NdfCP_A$) (Poultney et al., 2020).

2.4.5 Legume Nitrogen Recovery Efficiency

The nitrogen recovery efficiency (NRE) in the considered compartment was determined by the following formula:

$$NRE = \frac{NdfCP_x * N_x}{N_{C.ens}} \quad (3)$$

Where $NdfCP_x$ is the proportion of N in the considered compartment derived from *C. ensiformis* (%), N_x is the amount of N in the considered compartment (kg N ha^{-1}), and $N_{C.ens}$ is the amount of N supplied by the legume residues (kg N ha^{-1}).

N_x was calculated from the N concentration of the compartment and its dry mass (kg ha^{-1}). For sugarcane, as the N concentration of leaves does not represent the N concentration of the whole sugarcane, the N concentration was the mean N concentration of the aboveground biomass of the corresponding treatment, determined simultaneously on the whole plot. The same sample was used for the mulch and soil compartments to measure the N concentration and the ^{15}N abundance. The dry mass of sugarcane was determined on the whole plot using an allometric relationship for the sampling dates during the crop cycle and estimated from the four central rows x 3 m of the plot at harvest (see Chapter 2 - Section 2.4.2). The mass of the mulch was determined from the PVC ring. The mass of the soil per layer was determined by multiplying its volume with the bulk density.

2.5. Estimation of legume N loss by lixiviation

The soil moisture and mineral N were monitored in the SALSA trial to estimate the legume-N fluxes throughout the soil profile and the legume-N losses by deep drainage.

2.5.1. Soil water content measurements in ratoon 1

Soil water content (SWC cm^3 cm^{-3}) at depths 10, 20, 30, 40, 60, and 100 cm was measured using a frequency domain reflectometry sensor (PR2 – Profil Probe, Delta-T Devices Ltd., Cambridge, UK) each week during the first ratoon. Measurements were made in one tube in the soil per elementary plot located at a 40 cm distance from the sugarcane row.

2.5.2. Modeling of water fluxes in ratoon 2

A model based on Richard's equation for simulating one-dimensional water flow (Hydrus-1D) was used to quantify water drainage at different soil depths (Šimůnek and van Genuchten, 2008). The four soil materials implemented in Hydrus-1D were the 0-10 cm, 10-20 cm, 20-30 cm, and 30-60 cm soil layers. The soil hydraulic properties of these four materials (water retention and hydraulic conductivity) were estimated from the ROSETTA pedo transfer functions based on neural network analyses (Schaap et al., 2001). The van Genuchten parameters and Ks were thus calculated using textural distribution, bulk density, and two water retention points. These soil properties were characterized on the 0-20, 20-40, and 40-60 cm soil layers in a previous study (Jones et al., 2021, 2019). Bulk density was determined with 5 cm diameter sharp edge cylinders (98.17 cm^3) in the two soil layers. Water retention was measured at field capacity (33 kPa suction) and wilting point (1500 kPa suction) using Richards' apparatus (Richards, 1947).

The boundary conditions input was acquired from the nearest meteorological station and irrigation records. Daily precipitation and potential evapotranspiration were monitored and computed by a weather station located 10 m away from the study site. Evaporation was calculated using the cultural coefficient (Kc) of 0.4, 1.25, and 0.75, corresponding to initial, mid-season, and late-season sugarcane growth stages (Allen et al., 1998). In the legume treatment, the bean Kc of 0.33 was added by adjusting this value to the surface area covered (i.e., 2/3).

Evapotranspiration was partitioned into soil evaporation and plant transpiration fluxes based on the leaf area index (LAI). The LAI was measured in each plot with a Sunscan™ canopy layer each month in three positions: above the sugarcane canopy, above the companion plant, and on the soil. The Feddes (1982) root water uptake model was used for transpiration with Hydrus default parameters for Sugarcane.

The model gave water fluxes at 10, 20, 30, and 60 cm, considering 60 cm as the bottom output of the system. The model's ability to simulate the soil's water status was validated by comparing the simulated moisture content at different depths with the moisture content observed in the field using probes during the first ratoon. The model simulation of water fluxes in ratoon 2 was used to estimate N lixiviation.

2.5.3. Mineral N soil measurements in ratoon 2 and lixiviation estimation

The ammonium and nitrate concentrations were measured from the death of the legume in June 2021 to the sugarcane harvest. Near the three microplots, PVC rings were placed to follow the mineral soil N during legume decomposition at 0, 7, 14, 21, 35, 53, 84, and 100 days after legume restitution. The amounts of legume residues and sugarcane mulch placed in the PVC rings were the same as in the microplot experiments. The PVC rings had a diameter of 10 cm and a height of 10 cm. At each sampling date, soil samples were collected with a 5 cm diameter auger for the 0-10, 10-20, 20-30, and 30-60 cm layers. A 10 g aliquot was sampled for extraction with 40 mL 1 M L^{-1} KCl solution (soil: solution ratio 1:4).

Finally, nitrate fluxes were calculated at each date, and each depth was multiplied by mean concentrations in the soil solutions by the flux of gravitational water estimated from the Hydrus model. The lixiviation was considered as the nitrate fluxes at the deepest N soil measurements (60 cm).

2.6. Chemical analysis

The ^{15}N (biological N fixation and ^{15}N labeled legume experiment) and ^{13}C (litter bags experiment) isotopic abundance (atom%) were measured with an elemental analyzer (Vario ISOTOPE Cube, Elementar, Langenselbold, Germany) coupled to a gas isotope ratio mass spectrometer (IsoPrime 100, Elementar UK, Cheadle, UK), at the SilvaTec platform (UMR Silva, INRA Grand Est-Nancy, France), on samples ground into a fine powder in a ball mill (Cyclotec CT Sample Mill, Foss Tecator, Hillerod, Denmark).

Total C and N concentrations (aboveground and root of *C. ensiformis*, litter bags experiment, sugarcane in the ^{15}N labeled legume experiment) were measured with an elemental analyzer

(Vario Max Cube, Elementar Analyse Systeme GmbH, Hanau, Germany), and NH_4^+ and NO_3^- soil contents were determined by colorimetry at the CIRAD laboratory in La Reunion.

2.7. Statistical analyses

For the fixation data, Ndfa, N mass fixed, and total N mass of *C.ensiformis* were subjected to analysis of variance (ANOVA) with linear mixed effects models. The models included the plot as a random effect, irrigation treatment, nitrogen treatment, ratoon cycle, sampling date, and their interactions as fixed effects. For the litter bag data, the decomposition rate of the CP and SC compartments and the whole litter bag were subjected to analysis of variance. The models included the treatments and sampling date, and their interactions as fixed effects. Insignificant interactions were eliminated for each variable until best-fitting models were formed using the Akaike and Schwarz information criteria (AIC and BIC). A post hoc analysis of each mixed linear model was then realized using Tukey's HSD test. Box-Cox transformations were used when normality and variance assumptions were not respected. All analyses were performed in R 4.1.1 (R Core Team, 2021) using packages nlme (Pinheiro et al., 2021) and emmeans (Lenth et al., 2018) for tests of linear mixed effects model fits and for post hoc tests.

3. Results

3.1. Biological N fixation and N mass

Throughout the study, the *C. ensiformis* $\delta^{15}N$ values varied between -1.68 and 5.68 ‰ while the $\delta^{15}N$ values of the reference plants varied between 2.02 and 7.82 ‰. The difference in $\delta^{15}N$ between *C. ensiformis* and the reference plants varied from -2.15 to 6.67 ‰, highlighting some cases with no fixations.

The proportion of N derived from the atmosphere (Ndfa) of *C. ensiformis* varied widely among treatments and ratoon cycle, with an average of 53%. Ndfa was affected by W treatment ($P < 0.001$), N treatment ($P < 0.0001$), and ratoon cycle ($P < 0.0001$) but not by their interaction (Figure 1a). Ndfa was higher by 70% in the irrigated (W) treatment compared to rainfed (0W) and higher by 53% without N fertilization (0N) compared to N fertilization (Nopt) treatment. The higher Ndfa was observed for the combination W+0N with 78% of N fixed from the atmosphere. Ndfa also increased over a year of ratooning (Figure 1b).

The annual amounts of N fixed in the aboveground biomass of *C. ensiformis* ranged from 0 to 78 kg N ha^{-1} (Figure 1c). The amounts of N fixed in the aboveground biomass were affected by the W x N interaction ($P = 0.046$) and the interaction between the ratoon cycle and N treatment ($P = 0.002$), as well as the ratoon cycle ($P<0.0001$). On average, the highest amount of N fixed was found in W+0N (44 kg N ha^{-1}), higher than 0W+N (31 kg N ha^{-1}), and the lowest amount was found in both W+N and 0W+0N (17 kg N ha^{-1}, Figure 1c). The amount of N fixed was 8.3, 34.8, and 38.7 kg N ha^{-1} during the ratoons 1, 2, and 3, respectively, following the same pattern as Ndfa and the aboveground dry mass (see Chapter 2).

Finally, the total N mass in the legume (fixed + soil + roots) varied little between treatments (52.1 kg N ha^{-1} on average, Figure 1c) but varied with crop cycle with 27.9, 68.2, and 58.8 kg N ha^{-1} on average in ratoon 1, 2, and 3.

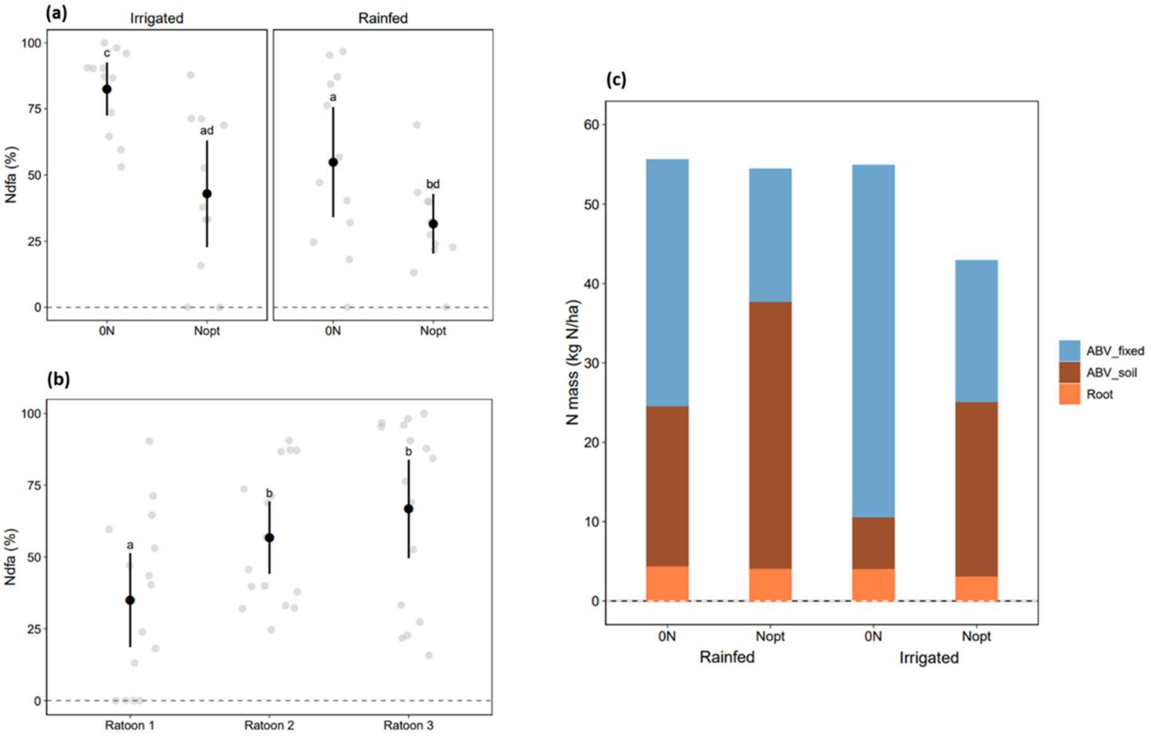

Figure 1. Nitrogen derived from the atmosphere (Ndfa) in the aboveground part of *C. ensiformis* measured at flowering, depending on the irrigation (irrigated or rainfed) and fertilization management treatments (no urea fertilization 0N or urea fertilization Nopt) (a) and the ratoon cycle (b). The total N mass in the legume, depending on fertilization and irrigation management, is presented as an average across ratoon cycles for aboveground and belowground components (c) with the distinction of the aboveground N mass originated from the atmosphere (ABV_fixed) or the soil (ABV_soil).

3.2. Sugarcane and legume residues decomposition and N release

Legume (CP) residues followed an exponential decomposition pattern (Figure 2a). The legume residues decomposed faster than sugarcane straw (SC) residues, with average decay rates in all mixtures of 3.05 and 1.19 year^{-1} for the CP and SC compartments, respectively. The mixture composition did not affect the decomposition rates in biomass of the CP (P = 0.53) and SC compartment (P = 0.67). After one month of decomposition, the CP compartment had lost, on average, 59% of its biomass, which can be related to the low C: N of the leaves (C: N = 11:1) representing 64% of the compartment CP. The remaining CP biomass was mainly stem tissues (observations; 32% of CP compartment, C: N = 31:1). The SC compartment had lost 23% of its mass after 1 month and 54% after 7 months (Figure 2a). Concerning the whole litter bag, mixtures showed differences in decomposition rates in biomass (P = 0.0012). The decay rates increased with the proportion of CP residues in the litter bag. At final sampling, the litter mass loss of the litter bag was 49, 65, 72, 73, and 83% for 100SC, 50CP/100SC, 100CP/100SC, 100CP/50SC, and 100CP mixtures (Figure 2a).

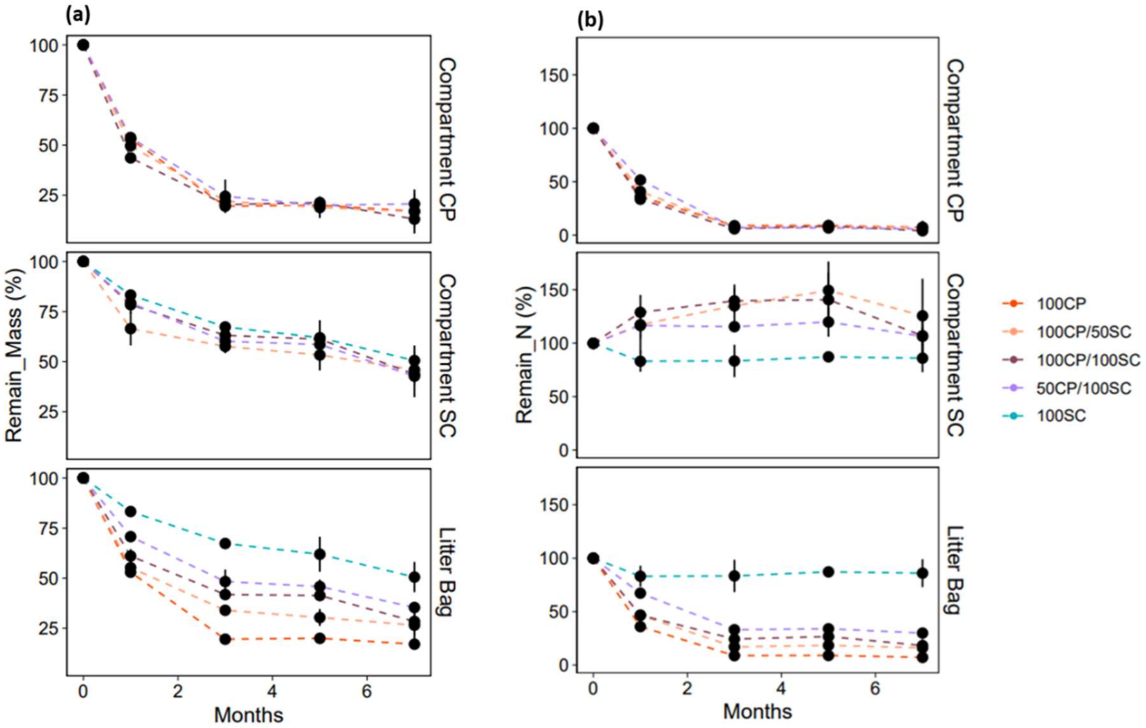

Figure 2. Change in biomass (Remain_Mass, a) and N mass (Remain_N, b) over time expressed as a proportion of the initial mass depending on litter bag mixture composition (see 2.3 for details). The change in biomass is represented for the legume compartment only (CP), sugarcane compartment only (SC) within the mixtures, and whole liter bags.

After 3 months of decomposition, the CP compartment had lost 93% of its N, and only minor N-loss changes were observed (Figure 2b). The SC residues contributed barely, if at all, to the release of N into the system during the experimental period. After 7 months of decomposition, almost 80% of the N initially present in the sugarcane mulch remained for the 100SC treatment (Figure 2b). In the other treatments, an increase in N concentrations in the SC compartment was observed as early as 1 month. This N would result from the immobilization of the legume's N on the sugarcane mulch. At the end of the experiment, the N amounts of the SC compartment were still superior to their initial values for the 100CP/50SC, 100CP/100SC, and 50CP/100SC treatments. Similarly to biomass, the rate of N release increased gradually with the proportion of legume in the mixture for the whole litter bags.

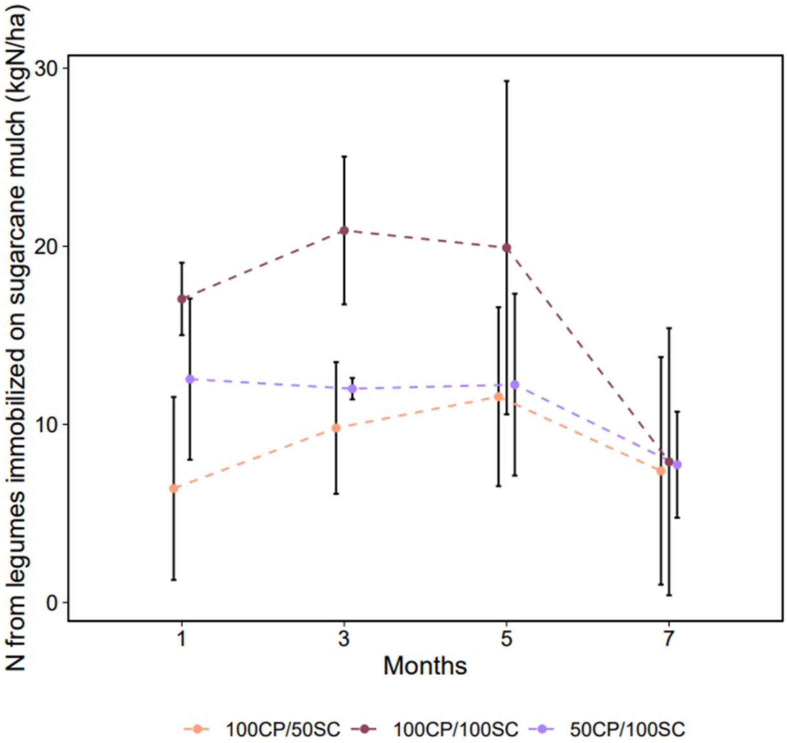

Figure 3. N from legume immobilized within the sugarcane mulch in the litter bags, depending on the mixture composition.

Based on the increase in N mass in the SC compartment, the quantity of N from legume immobilized on sugarcane mulch was estimated between 6 to 17 kg N ha^{-1} after 1 month, depending on mixture composition (Figure 3). At the end of the experiment, an average of 7.7 kg N ha^{-1} was still immobilized on the sugarcane mulch, representing 4.5% of the N initially present in the CP compartment. In the SALSA trial during ratoons 1, 2, and 3, we were in a situation of 53CP/100SC at the legume's death, close to the 50CP/100SC mixture.

3.3. Legumes N recovery in sugarcane and soil

In this experiment, we were in a situation of 57CP/100SC for the first experiment (2020-2022) and 60CP/100SC for the second experiment (2021-2022), close to the 50CP/100SC mixture. For the two years, the loss of N from legumes follows a similar pattern to that observed in the litter bag experiment.

One month after the deposition of ^{15}N-labeled legume residues, 49% of the legume N was lost from the soil-plant system in 2020 and 43% in 2021 (Figure 4). Most of the legume N was recovered in the mulch and the topsoil layer, with an average NRE of 31% and 12% for the mulch and the 0-5 cm soil layer, respectively, across years. At harvest, i.e., 4 months after the deposition of ^{15}N-labeled legume residues, 23% of the N remained in the mulch and 24.5% in the 0-5cm soil layer, with similar values in 2020 and 2021.

The sugarcane recovered a smaller proportion of the legume's N, with an NRE of 7% on average at harvest (4 months after the deposition, Figure 4), representing only 3% of its total nitrogen mass. During the subsequent ratoons, the sugarcane recovered a similar percentage of N from legumes, with an average NRE of 5.5% at harvest n+1 and 7.4% at harvest n+2 (Table S1). The nitrogen derived from the legumes in sugarcane still represented only a negligible proportion of its total nitrogen mass, with a value of nitrogen derived from legumes of 1% on average at harvest (n+1 and n+2).

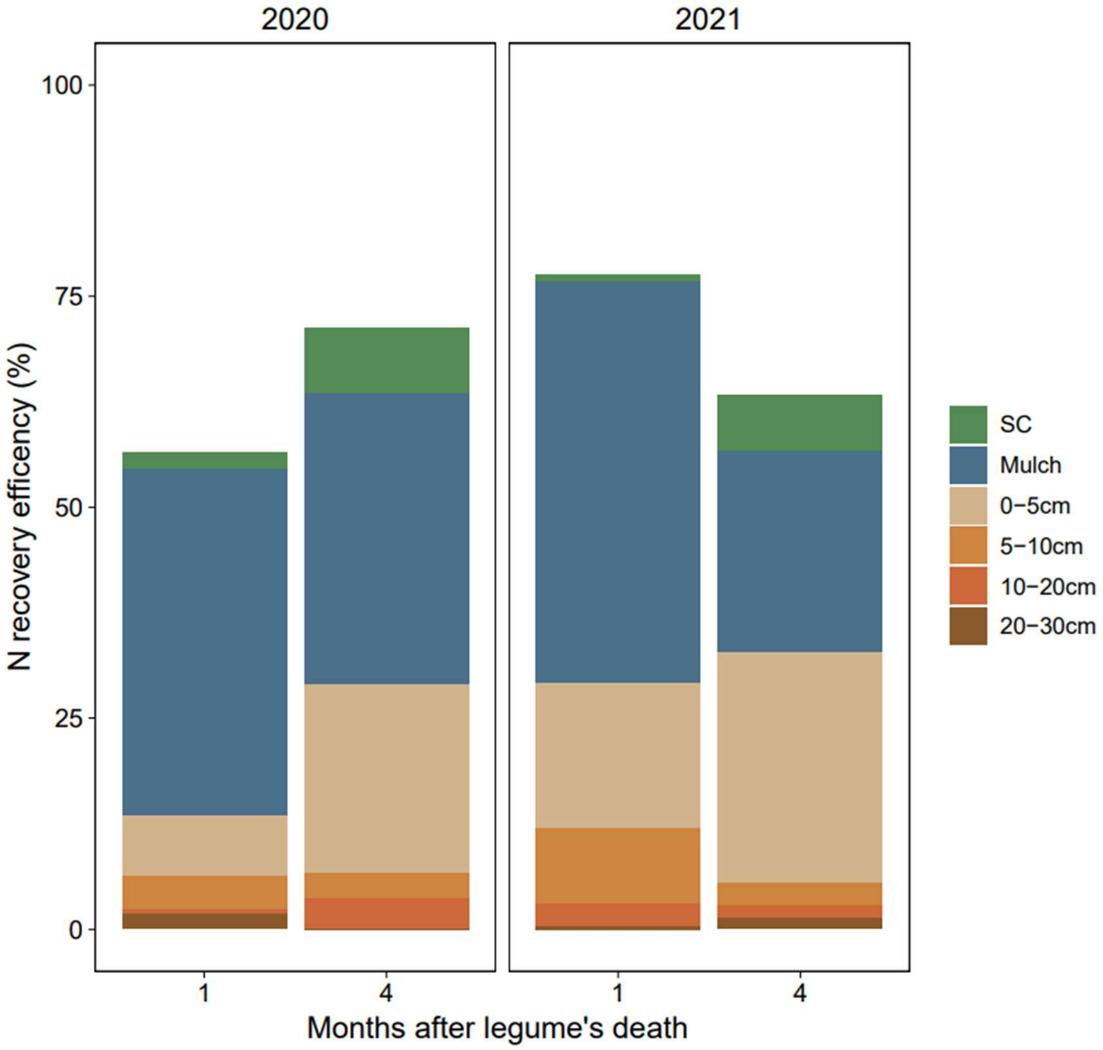

Figure 4. Nitrogen fate of the legume expressed as N recovery efficiency (%) in the different compartments (sugarcane: SC, mulch, 0-5, 5-10, 10-20, and 20-30 cm soil layers) one month after legume death (9 months after sugarcane harvest), or 4 months after legume death (sugarcane harvest) of the two experiments (2020-2022 and 2021-2022).

3.4. NO_3 fluxes

One week after the deposition of the legume residues, there was a slight peak in nitrate concentration in the topsoil at 0-10 cm with 3.61 mg L^{-1} in the SC + CP treatment, whereas the N concentration was 1.14 mg L^{-1} for the SC treatment (Figure 5b). This difference between the two treatments persisted two weeks after the deposition of the legumes. The nitrate concentration remained the same between the two treatments after this point. There was no difference between the treatments at lower soil depths. The nitrate concentration had a similar pattern at the lower soil depths to that at 0-10 cm but with lower concentrations and variations over time.

We used a modeling approach to estimate drainage and nitrate leaching. Concerning the calibration of the Hydrus model on soil water stocks in the first year, the Hydrus model poorly represented water content boundaries in the surface horizon (0-10 cm), but it did represent well soil water dynamics in the rest of the soil profile, i.e., between 10 and 60 cm (Figure S1). The model enabled us to simulate water stocks not measured in the second year (Figure S1).

During the period of interest, i.e., from legume death in June 2021 to harvest in October 2021, the model simulated drainage in the 30-60 cm layer with increase around 22, 30, and 80 days after legume deposition, for a cumulated drainage of around 150 mm 100 days after legume death (closed values between SC and SC+CP treatments, Figure 5c). This drainage did not result in the significant loss of N through nitrate leaching. Indeed, a negligible amount of nitrate transited to the 30-60 cm layer with 0.87 kg N ha^{-1} in the SC treatment and 0.33 kg N ha^{-1} in the SC + CP treatment (Figure 5d).

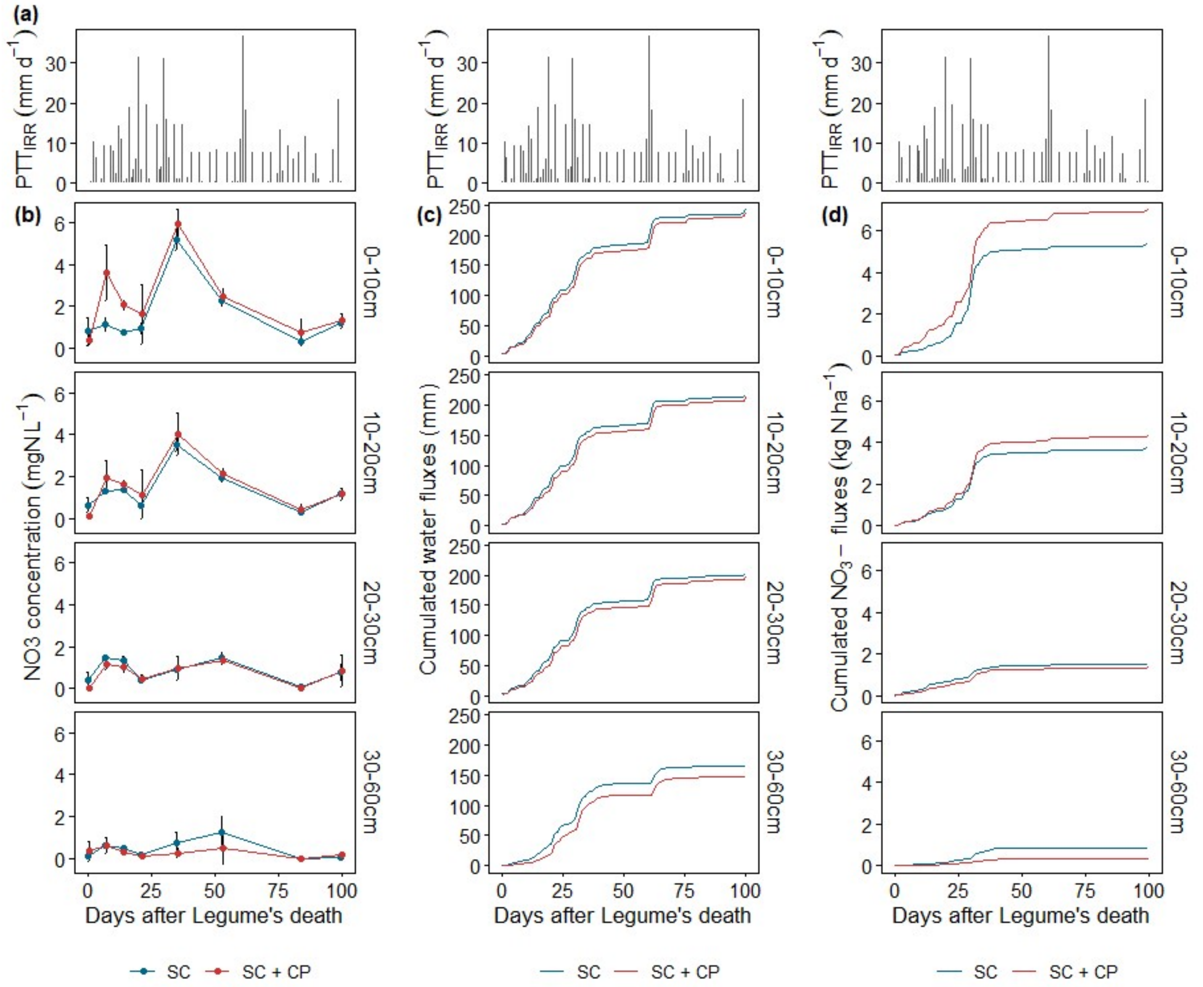

Figure 5. Dynamics of rainfall + irrigation (PPTIRR, a), measured NO3^{-} concentration (b), cumulated water fluxes (c), and cumulated NO3^{-} fluxes (d) after legume death in the 0-10, 10-20, 20-30, and 30-60 cm soil layers, depending on the absence (sole sugarcane – SC, blue) or presence of legume (SC + CP, red). The simulations were performed with the Hydrus-1D model.

3.5. Legume nitrogen fate balance

Nitrogen flows through legumes averaged over the three years were estimated (Figure 6). In addition to the legume, three compartments were considered: soil, mulch and sugarcane. The average amount of nitrogen fixed by the legume (19 kg ha^{-1} yr^{-1}) was equivalent to the amount of nitrogen lost by the system during its degradation. The amount of nitrogen taken up by the legume from the soil (22 kg ha^{-1} yr^{-1}) was equivalent to the nitrogen returned to the soil or immobilized in the sugarcane mulch. Finally, the amount of nitrogen taken up by the sugarcane during its growth was equivalent to the amount returned after harvest.

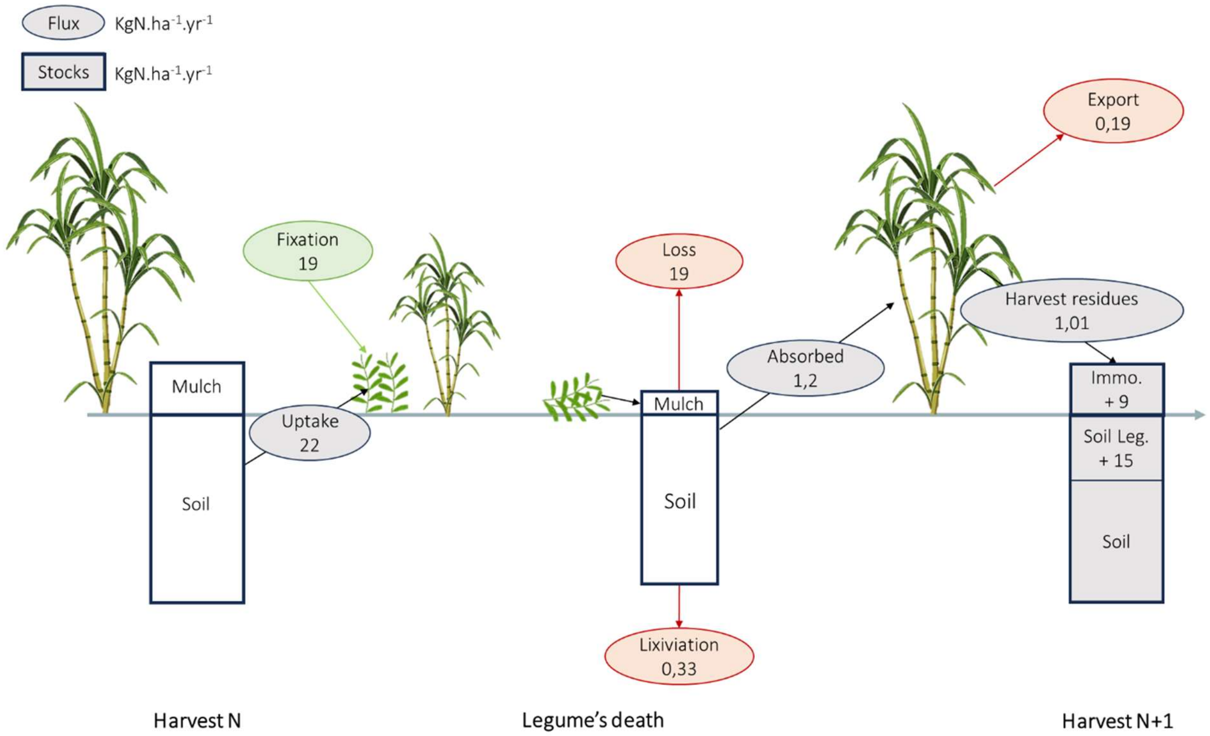

Figure 6. Balance of nitrogen flows through legume average over the three years of measurements between two sugarcane harvests, expressed in kg ha^{-1} yr^{-1}.

4. Discussion

4.1. Limited nitrogen input from legume fixation

4.1.1. The effect of growth conditions on the fixation rate of nitrogen

On average, about half of the N accumulated in the legume was derived from BNF, which is lower than the values reported in the literature for *C. ensiformis* (e.g., 71 to 90% in Córdova-Gamas et al., 2016; de Resende et al., 2003). This proportion varied widely among treatments and ratoon cycles, indicating that growth conditions had limited the process of symbiotic N_2 fixation. The first factor to explain this variability was the N availability, with the %Ndfa almost 50% higher without N fertilization. The inhibition of symbiotic N_2 fixation due to high N input or high soil N fertility has been reported in many studies (Andersen et al., 2005; Hauggaard-Nielsen et al., 2001). These results suggest that N application rates must be tailored carefully to balance maximizing BNF and avoiding sugarcane yield penalties.

The %Ndfa was also lower by 32% in the rainfed treatment compared to the irrigated treatment. The importance of sufficient water supply to ensure BNF has been reported in previous studies (Pandey et al., 2017; Santachiara et al., 2019). Water stress affects the formation, size, and activity of the nodule, with some evidence that it is particularly deleterious during the early stages of development when the nodules are not completely established (King and Purcell, 2005; Serraj et al., 1999).

We observed an increase of %Ndfa over the three ratoon cycles. This result might be related to concomitant effects. First, it could be due to the reference plant, which changes for the third ratoon cycle. Indeed, the $\delta^{15}N$ moyen of *G.abynssinica* (ratoon 1 and 2) was higher than that of the $\delta^{15}N$ of weeds (ratoon 3) ($p=0.013$). Nonetheless, the $\delta^{15}N$ of *G.abynssinica* was not different between the first and the second ratoon ($p=0.61$), while the most pronounced increase was observed between these two ratoons. Thus, the increase between ratoon 1 and 2 might be related to greater strength between host and rhizobium, favoring BNF. Several studies showed that the nodulation process was positively correlated to the rhizobial population (Ballard et al., 2004; Mothapo et al., 2013). It has been demonstrated that the soil-native rhizobial population increased after the re-introduction of the legume host in the agroecosystems (Brockwell et al., 1987; Venkateswarlu et al., 1997). The continuity of intercropping over the cycles seems important to maximize the benefit from the nitrogen-fixing capacity of legumes. A second explanation is that the increase of BNF over the three ratoon cycles is related to a decrease in N availability over the years. Indeed, tillage at sugarcane planting increases soil organic matter's mineralization (Balesdent et al., 1990), which fades after a few cycles. This hypothesis is strengthened by a more pronounced increase in BNF over the years in the unfertilized treatment (Figure S2), as well as an increasing nitrogen stress for sugarcane growth over ratoon years (see Chapter 2).

The method used to estimate the BNF is a good proxy, but several biases can lead to substantial error in the estimate of %Ndfa (Unkovich et al., 2008). The value of %Ndfa is very sensitive to the exact value of β and the $\delta^{15}N$ of the reference plant. In this study, β was not determined. This value is difficult to determine as it can vary with growth stage, rhizobium strain, and the proportion of total plant N in the aerial tissue (Boddey et al., 2000). We used the lowest detected $\delta^{15}N$ in the field as β (as done in previous studies: Motisi et al., 2007; Oberson et al., 2013), meaning that the BNF may have been over-estimated if the actual value of β for *C. ensiformis* is more negative. The reference plant represents the $\delta^{15}N$ of the soil N available to the legume (Unkovich et al., 2008). The method assumes that the legume species and the reference plant have a similar root distribution and soil N acquisition (Unkovich et al., 2008). However, the root behavior is very different between species. We used a reference plant (*G. abyssinica*) with a similar root system (i.e., taproot system) and rooting depths as *C. ensiformis*, and the two species were exposed to the same soil and fertilizer conditions. This is the best method to use with the reference plant approach (Unkovich et al., 2008; Unkovich and Pate, 2000). As the reference plant did not germinate during the third ratoon, we used spontaneous plants instead, which might have induced a bias as the root behavior and the germination time were unknown. The different $\delta^{15}N$ signatures of *G. abyssinica* and weeds can result from different isotopic discrimination among the species or extraction from different rooting depths. The difference can also be related to differences in soil water content between years and associated differences in N (soil and fertilizer) losses (Unkovich et al., 2008).

4.1.2. The N inputs are related to mass accumulation.

The estimated annual N input through symbiotic fixation was 23 kg N ha^{-1} on average across treatment and years, which is in the lower range of the values reported in sugarcane intercropping systems (Ambrosano et al., 2013; Córdova-Gamas et al., 2016; de Resende et al., 2003; de Souza Filho and de Andrade, 1985; Prellwitz and Coelho, 2011). It corresponds to 17% of the annual N inputs by fertilizers and, on average, 18% of the total N accumulated in the sugarcane aboveground biomass during its crop cycle. However, this estimate does not account for belowground N inputs through rhizodeposition and root mortality/restitution, which can represent 17 to 74% of the total legume N (Wichern et al., 2008). Considering that the amount of legumes N rhizodeposition is 3.4 times N accumulated in the roots, as reported by Wang et al., (2021), the belowground N inputs could represent up to 22 kg N ha^{-1} in our system (assuming that $\delta^{15}N$ of the rhizodeposition and roots is the same as the aboveground compartment).

The aboveground biomass was an important factor in determining the amount of N fixed by the *C. ensiformis*. The importance of legume biomass on the total BNF has also been reported in other studies (Oberson et al., 2013; Pandey et al., 2017). N mass accumulation is limited in such additive intercropping systems as the sowing surface of the legume is reduced (53% less than in sole crop in our case), which largely explains the large differences between N inputs in rotation and intercropping. Besides, the growth of the legume is constrained by competition for resources with the sugarcane. As a comparison, the N accumulation of *C. ensiformis* as a sole crop was 220 kg N ha^{-1} on average in a trial conducted in 2021 on the same experimental station as the SALSA trial (data not published). For the same area sown, the N mass in intercropping was 50% lower than in the sole crop. Thus, an essential key to increasing total BNF is the capacity of the legume to accumulate N. In the SALSA trial, the sugarcane productivity was favored with a late sowing date of the legume but at the expense of the legume's growth, which can explain the low N inputs here. Indeed, the sowing date of the legume can significantly impact the legume's growth and N accumulation. For example, in Prellwitz and Coelho (2011), the N mass of *Crotalaria juncea* intercropped with sugarcane was three-fold higher for early sowing compared to late sowing.

4.2. Can legumes sustain soil N fertility?

4.2.1. Sugarcane mulch can be a sink for legume-N due to microbial immobilization.

We estimated that, on average, 14 kg N ha^{-1} was immobilized on the sugarcane mulch during the first five months of decomposition. However, this value varied among treatments from 6 kg N ha^{-1} to 21 kg N ha^{-1}. We observed that the source (legume) and the sink (sugarcane mulch) impacted the amount of N immobilized on the mulch. Compared to the 100CP/100SC treatment, reducing the proportion of sugarcane mulch in the mixture (100CP/50SC) reduced the amount of N immobilized on the mulch by 53%. It suggests that the N release from the legume residues exceeded the microbial population's capacity to retain N on the sugarcane mulch. This result aligns with a study by Frimpong et al. 2011). They observed that increasing the proportion of high C: N maize residue in a maize-cowpea mixture resulted in increased immobilization of cowpea N. Conversely, reducing the proportion of legume in the mixture (50CP/100SC) reduced the amount of N immobilized on mulch by 36%. This is consistent with Recous et al. (1995), who demonstrated that immobilization of N from maize residues could increase with the increasing availability of N to the decomposers.

Concomitantly to legume N immobilization, a faster decomposition of the sugarcane compartment was observed in mixed litter bags compared to single sugarcane bags. This finding is consistent with the hypothesis that altered decomposition rates (non-additive effect) in litter mixture are related to the transfer of N from the N-rich component to the N-poor component (Berglund and Ågren, 2012). This interactive effect is expected to vary depending on the heterogeneity of chemical traits in the mixture (Redin et al., 2014) and the ratios of the component residues (Liu et al., 2010). However, this acceleration in the decomposition of sugarcane mulch remained limited.

Even though the immobilization of N in the microbial biomass may be undesirable for sugarcane nutrition, it may provide an initial barrier against N losses and a mechanism for long-term incorporation into soil organic matter. Previous studies in tropical intercropping systems showed that mixtures of legume and low-quality residues improved C and N sequestration and decreased losses relative to the sole addition of legume residues (maize-cowpea in Ghana in Frimpong et al. (2011); cotton-cowpea in Zimbabwe in Rusinamhodzi et al. (2009)).

4.2.2. Immobilized legume-N ends up in the soil without stock gain.

The ^{15}N recovery in the soil was an average of 31% after 4 months of decomposition. The mineral N data indicate that only a small proportion of soil mineral N was derived from residues. Therefore, it is likely that most legume-N reaching the soil compartment has been incorporated into the soil organic N pool as living soil microbial biomass, microbial residues, or recalcitrant organic forms. Very few studies using ^{15}N labeled legume residue investigate the ^{15}N recovery in the soil compartment (Gardner and Drinkwater, 2009), and most of the studies concern buried residues. The ^{15}N recovery values from legume residues in the soil reported in the literature range from 38 to 81% under field conditions (Harris et al., 1994; Hemwong et al., 2009; Jensen, 1996, 1994; Sakonnakhon et al., 2005; Seo et al., 2006). In our study, the ^{15}N recovered in the soil falls below this range due to significant losses. However, this value does not consider the N still present in the mulch (23%). After 4 months of decomposition, the remaining legume-N primarily was either immobilized in the sugarcane mulch or the more recalcitrant fraction of the legume residues (i.e., stems with C: N = 31:1). We can assume that these two pools are less susceptible to losses and will be incorporated progressively in the soil organic matter during the following months.

The high mineralization rate in our tropical conditions and the low C:N ratio of *C. ensiformis* can account for the low ^{15}N recovery in the soil compared to the studies mentioned above. Indeed, in a study encompassing 13 experimental sites in diverse agroecosystems and climatic zones, Dourado-Neto et al. (2010) observed that increasing annual rainfall or temperature between sites resulted in a noticeable and highly significant reduction in the recovery of ^{15}N residues in the soil. In a meta-analysis, Gardner and Drinkwater (2009) found that the amount of N inputs explained 57% of the variability in unrecovered ^{15}N for labeled legume N sources, with losses proportional to the N inputs. The studies cited above reported a low recovery in the soil, associated with important losses, mainly when there was an asynchrony between the release of N from residues and the N demand of the subsequent crop. For example, in a sugarcane rotation with fallow legumes, Hemwong et al. (2009) observed that the slower decomposition of soybean (C: N = 33:1) resulted in better conservation of soybean-^{15}N in the system (total recovery = 80%) compared to groundnut (C: N = 21:1), which decomposed very quickly (total recovery=43%). The ability of legume companion plants to sustain and build up the N reserve of the SOM is, therefore, strongly linked to the mineralization rate of residues. Future studies need to focus on management practices that improve the synchrony between N supply from residue mineralization and sugarcane demand, as well as reducing losses through leaching and volatilization.

The nitrogen incorporated into the soil may be more or less available for subsequent crops, depending on the degree of microbial processing and interactions with the soil mineral constituents (Bending and Turner, 1999). Further studies are needed to assess whether the legume N recovered in the soil is stabilized in the long term or will be available for subsequent growth cycles.

4.3. Low contribution to sugarcane nutrition, most likely due to the emission of reactive nitrogen into the atmosphere

4.2.1. Sugarcane nutrition

The direct contribution of aerial legume biomass to sugarcane nutrition was meager, with only 3% of sugarcane-N derived from *C. ensiformis* residues. Several studies showed that %NdfCP was related to the N input amount and the subsequent crop's total N uptake (Arcand et al., 2014; Hood et al., 1999; Mayer et al., 2003). In our study, the particularly low %NdfCP is exacerbated by the important N mass of sugarcane compared to other crops and the relatively low N mass of the legume companion plant.

On average, 7% of legumes ^{15}N was recovered in sugarcane at harvest the first year of application (i.e., 4 months after the deposition of ^{15}N-labelled legume residues). The contribution of legumes to crop nutrition is often small, particularly under field conditions with ^{15}N recovery legume residues usually less than 20%: 2-3% of soybean and groundnut residue-N recovered in sugarcane (Hemwong et al., 2009), 22% of pea residue-N recovered by canola (Arcand et al., 2014), 8-12% of pea residue-N recovered in wheat and canola (Mayer et al., 2003), 11% of soybean residue-N recovered in rice (Norman et al., 1990), 6% of groundnut residue-N recovered in maize (Sakonnakhon et al., 2005). Low ^{15}N recovery by crop from labeled legume residues can be attributed to a combination of immobilization after residue N release and N losses.

The ^{15}N recovery in the sugarcane may also have been underestimated due to "pool substitution". This theory suggests that a portion of the ^{15}N applied is immobilized in the microbial biomass or adsorbed in the form of $^{15}NH_4$ on the mineral surface, coinciding with an early release of ^{14}N from the microbial biomass or desorption of $^{14}NH_4$ (Hart et al., 1986; Jenkinson et al., 1985). This phenomenon would result in a lower ^{15}N recovery in the crop. However, we hypothesized that the tropical conditions allow for the rapid remineralization of immobilized ^{15}N as well as rapid nitrogen exchanges within the soil-plant system, which would reduce the impact of pool substitution on ^{15}N recovery in sugarcane.

Over three crop cycles, the cumulative uptake of legume residue-^{15}N by sugarcane was 21% of the applied residue N, with an average of 7% per ratoon cycle. This result is similar to those reported in the literature (from 2 to 8% of legume residue-N recovered in the second and third subsequent crop: Jensen, 1996; Kumar et al., 2001; Ladd and Amato, 1986). Although an annual contribution of 7% of the N supplied by legumes is relatively small compared to the requirements of sugarcane, it reflects the importance of SOM in supplying N to the crop. In La Reunion, an average of 69% of the N accumulated by sugarcane comes from the soil (Ramos, 2023) emphasizing the ongoing significance of maintaining sufficient SOM content, with crop residues potentially playing a vital role in this process.

4.2.2. N losses from the mulch-soil-plant system

Losses occurred very quickly after the death of legumes. Almost half of the legume N was not recovered in the mulch-soil-plant system one month after the deposition of ^{15}N-labelled legume residues. Several studies reported significant losses through leaching from legume residues (Frimpong et al., 2011; Nyawade et al., 2020; Sakonnakhon et al., 2005). In our case, leaching cannot explain the low recovery of legume residues. Indeed, the mineral N data showed higher N availability in the SC + CP treatment compared to the SC treatment. However, this difference between the two treatments was minor and was only observed in the topsoil at 0-10 cm. The estimated N flux confirmed that both treatments had almost no leaching losses. The low losses through leaching were certainly linked to the period of residue mineralization (i.e., 8 months after harvest). The sugarcane and its rooting system are sufficiently developed at this time, allowing a rapid uptake of the available N in the soil (Poultney, 2021). A second explanation is linked to the characteristics of the soil. In a study conducted at the same experimental site, Poultney (2021) observed that leaching was very low, even with significant urea or organic fertilizer inputs. They attributed this result to the high anion exchange capacity of the soil at the study site. Tropical soils commonly have a high anion exchange capacity, which reduces nitrate transfer within the soil profile (Feder et al., 2020).

A part of the N losses from legume residues could be linked to N_2O emissions. Few studies measured N_2O emission from the decomposition of legume residues left on the soil surface, with values reported around 2% of the N content of the legume: 1.75% for vetch (Schmatz et al., 2020), 1% for alfalfa (Larsson et al., 1998), 3.78, 3.29 and 1.97% for jack bean, velvet bean, and sunn hemp respectively (Sant'Anna et al., 2018). Generally, higher N_2O emissions are observed for crop residues with a high N content (low C:N ratio) (Huang et al., 2004; Sant'Anna et al., 2018). Besides, crop residues left on the soil surface can create "hot spots" of denitrification at the soil-mulch interface (Kravchenko et al., 2017; Oliveira et al., 2023). However, in our case, we supposed that the quantities of N_2O emissions were low due to the small amount of N accumulated by the legume (Sant'Anna et al., 2018).

Part of the ^{15}N-labeled legume residues might also have been transported below the sampled soil depth or lost due to translocation within roots to outside of the microplots or transported outside of the plots by the macro-fauna. However, we assume this accounts for only a minor portion of the unrecovered ^{15}N.

At the end of the crop year, on average, 39% of the legume-N remained unrecovered. Previous studies attempting to measure the N budget of systems with legume green manures reported significant N losses that could not be explained by leaching or crop removal (Ferreira et al., 2015; Hemwong et al., 2009). The literature reports a wide range of NH_3 losses from legume mulch, ranging from less than 3 to 47% of the N content of the crop (de Ruijter et al., 2010; Glasener and Palm, 1995; Larsson et al., 1998). These values fall beneath the losses we attribute to volatilization in our investigation. Nonetheless, these studies were mainly conducted in temperate or dry climates or under controlled conditions, and several factors can support higher values of NH_3 emissions in our conditions. In our tropical context, the combinations of high temperatures and air humidity can have promoted residue mineralization and thus enhanced NH_3 emissions. Whitehead et al. (1988) observed that NH_3 volatilization from ryegrass was under 1% of the plant N under dry conditions, while this percentage could escalate to 47% of the plant N if the residue decomposed over 70 days in humidified and saturated air. Similarly, He et al. (1999) reported that NH_3 volatilization increased two-fold with an increase in the incubation temperature from 5 to 25 °C. Our experimental site is highly exposed to winds (Ramos, 2023), which also promotes the volatilization of ammonia (Smith et al., 1996). The decomposition of legumes occurred during the dry season, characterized by intermittent rains. This likely prompted mineralization and NH_3 volatilization, but the amounts of rain or irrigation may not have been sufficient to incorporate N release from legume residues into the soil (Oliveira et al., 2023). Finally, the sugarcane mulch can have further exacerbated the N volatilization of the legume residues due to the restricted movement of newly mineralized N of the legume residues into the soil like observed with urea (Oliveira et al., 2023; Pinheiro et al., 2018).

5. Conclusion: The imperative to cut off losses.

Our results suggest a limited benefit of legumes regarding soil fertility and sugarcane nutrition. Indeed, legumes contributed only to 3% of the N accumulated by sugarcane during its growth. Furthermore, the amount of N_2 fixed is equivalent to the N losses from the soil-plant system during the decomposition of legume residues. The sugarcane mulch has a dual antagonistic effect in this regard. It acts as a sink for legume-N and increases losses through volatilization. The significant N losses observed in our study, linked to a combination of factors favorable to ammonia volatilization, highlight the need to better understand the N cycle intercropping systems of legumes and Gramineae in no-till with mulch management, which are promoted in tropical regions. The high mineralization rate can make it a major challenge to significantly reduce N losses in these regions. Future studies are needed to resonate the management of surface-mulched mixed residues to conserve the residue's nutrients in the soil-plant system.

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Conclusions générales et Perspectives

L'objectif principal de la thèse était de caractériser les interactions de compétition et de facilitation entre canne-à-sucre et légumineuse. Cette conclusion finale s'articule autour des points suivants : 1. La synthèse des principales conclusions de la thèse à travers les hypothèses de recherches formulées dans l'introduction générale. 2. Les perspectives de recherches qui découlent de ce travail. 3. Les utilisations potentielles de ces résultats pour accompagner l'évolution de la filière canne-à-sucre à la Réunion dans la réduction de sa dépendance aux intrants chimiques (herbicides et engrais azoté). Un schéma conceptuel des connaissances, sur les processus régissant les interactions canne x légumineuse x adventices, acquises au cours de cette thèse (trois chapitres et articles complémentaires écrits en co-auteurs et présentés en annexe) est présenté en Figure 1.

1. Impact des légumineuses sur la croissance et le rendement de la canne-à-sucre

***Hypothèse 1** : La compétition d'une plante choisie est moins forte que celle d'une communauté d'adventices ayant des stratégies d'acquisition des ressources différentes et complémentaires.*

Nous avons pu valider cette première hypothèse au cours de notre méta-analyse (Chapitre 1) en utilisant les mesures de couverture du sol par les adventices et les légumineuses effectuées dans les essais menés à La Réunion comme un indicateur de leur biomasse. Notre analyse montre que les adventices ont un impact sur le rendement de la canne-à-sucre deux fois plus important que celui des légumineuses. Ce résultat renforce ainsi l'intérêt d'utiliser des légumineuses de services pour réduire l'utilisation d'herbicides.

***Hypothèse 2 :** L'insertion d'une légumineuse en intercalaire de la canne-à-sucre modifie la disponibilité des ressources du milieu, ce qui peut affecter la croissance de la canne-à-sucre (et des adventices), et in fine son rendement.*

Pour vérifier cette seconde hypothèse, nous avons mobilisé le dispositif en station expérimentale. Nos résultats suggèrent que les légumineuses ont un impact indirect sur la disponibilité en ressources du sol pour la canne-à-sucre en raison d'une diminution du volume de sol accessible par les racines, plutôt qu'une compétition résultant d'une diminution de la quantité de ressource dans le sol. En effet, la biomasse racinaire de la canne-à-sucre diminue en présence de légumineuses (Chapitre 2), ainsi que la longueur de racines fines (Graillot, 2022, stage master sur le même essai) qui pourrait résulter d'une stratégie d'évitement par les racines de la canne-à-sucre, comme observé dans une étude complémentaire à cette thèse menée lors d'une année de plantation (Christina et al., 2023).

Cette réduction du volume de sol exploré par les racines a pour conséquence une diminution des ressources disponibles, ce qui affecte la nutrition azotée de la canne-à-sucre et entraîne une réduction de la biomasse aérienne due à une diminution du tallage (Chapitre 2). Notre étude montre également que l'allocation de la biomasse n'est pas modifiée en réponse à la compétition exercée par les légumineuses, indiquant une faible plasticité de la canne-à-sucre pour ce qui concerne le R/S ratio. Ainsi, l'impact négatif des légumineuses sur le rendement en tige de la canne-à-sucre résulte d'une réduction globale de la biomasse de la canne-à-sucre.

Au contraire de l'azote du sol, nous n'avons pas observé d'impact direct de la présence des légumineuses sur le contenu en eau du sol (Chapitre 2). Toutefois, il est important de noter que nos mesures ont été effectuées pendant un cycle de culture caractérisé par des précipitations abondantes. De même, notre méta-analyse suggère que l'impact négatif des légumineuses sur les rendements canniers est plus importants dans les contextes avec peu de précipitations (Annexe Chapitre 1). Des études supplémentaires seraient à mener dans des conditions plus contraintes pour mieux quantifier cet effet.

Cette thèse n'a pas pu mettre en évidence le fait que les légumineuses affectaient la croissance des adventices à travers une modification de la disponibilité en ressource. Néanmoins, des travaux complémentaires à cette thèse menés sur des réseaux d'essai à La Réunion ont montré que la capacité de contrôle des adventices était directement liée au développement des plantes de service (en association avec de la canne à sucre, Soulé et al., under review ; ou en pure, Christina et al., 2021; Négrier et al., 2023).

Hypothèse 3 : *Les interactions entre les espèces dépendent des dynamiques de croissance aérienne et racinaire des espèces, influencés par la disponibilité des ressources.*

Pour vérifier cette troisième hypothèse, nous avons utilisé à la fois le dispositif en station expérimentale et la méta-analyse, ce qui a permis d'obtenir des informations complémentaires sur l'influence de la disponibilité des ressources sur ces interactions. Les résultats montrent que la compétition entre les deux espèces est bidirectionnelle et dépend du développement de chacune d'elles. En effet, la compétition exercée par la légumineuse sur la canne à sucre est proportionnelle à son développement (Chapitre 1), lequel est lui-même corrélé à la quantité de lumière reçue, modulée par la croissance aérienne de la canne à sucre (Chapitre 2). Dans le cadre de notre itinéraire technique, où il y avait un décalage entre la coupe de la canne à sucre et le semis de la légumineuse, la croissance initiale de la canne à sucre a joué un rôle déterminant dans l'intensité de cette compétition réciproque. En termes d'impact sur la légumineuse, les résultats du Chapitre 1 suggèrent que l'intensité de la compétition pour la lumière exercée par la canne-à-sucre sur la légumineuse est d'autant plus marquée lorsque la distance entre les rangs de canne-à-sucre était réduite.

La compétition entre les deux espèces est fortement liée à la disponibilité de la ressource eau (précipitations et irrigation) dans notre dispositif expérimental, ce qui conditionne également l'offre en azote du sol. Ainsi, le chapitre 2 a illustré comment un stress azoté de la canne plus important pouvait s'observer en présence de légumineuse en condition de minéralisation du sol limitante. De plus, les résultats du chapitre 1 montrent également que la compétition entre les deux espèces est liée à la fertilité du sol et à la température, ces facteurs étant les principaux déterminants de la fourniture du sol en azote.

Hypothèse 4 : *La direction et l'intensité de la compétition peuvent être influencées par certaines techniques culturales : irrigation, fertilisation, date de semis et de destruction du couvert.*

Pour vérifier cette quatrième hypothèse, nous avons utilisé la méta-analyse et le dispositif en station expérimentale. Le chapitre 1 met en évidence que la date de semis et de destruction sont des facteurs clés pour diriger et moduler la compétition entre les espèces. Les résultats montrent qu'un semis tardif de la légumineuse favorise la compétitivité de la canne à sucre, et que la destruction précoce des légumineuses permet également de limiter l'impact sur le rendement de la canne à sucre.

Dans notre dispositif expérimental (Chapitre 2) nous avons observé que la disponibilité en eau, en particulier en début de cycle, influence de manière significative l'intensité de la compétition entre les deux espèces. De plus, dans la méta-analyse, nous avons observé que le rendement en canne avait tendance à augmenter avec les précipitations en conditions pluviales. Par conséquent, le pilotage de l'irrigation en début de cycle de culture est un facteur clé pour assurer le départ de la croissance de la canne à sucre et limiter l'impact des légumineuses sur le rendement en canne.

Au contraire, nous n'avons pas identifié d'effet significatif de la fertilisation azotée sur la direction et l'intensité de la compétition entre les deux espèces que ce soit dans la méta-analyse ou dans l'approche en station. Toutefois dans la métanalyse seul l'augmentation du carbone du sol est liée à l'augmentation du rendement relatif de la canne à sucre. Cette teneur plus élevée en carbone du sol pourrait être liée à une fertilité plus élevée du sol mais les données traitées ne permettent pas de conclure avec certitude sur ce lien.

Ce premier volet de la thèse a contribué à l'enrichissement de nos connaissances sur le partage des ressources et son impact sur le rendement de la canne -à-sucre dans ces systèmes multi-espèces. La réponse du système racinaire de la canne-à-sucre due à l'introduction des légumineuses mérite une attention particulière dans de futurs travaux de recherche. La méta-analyse et le dispositif en station expérimentale ont mis en lumière des options au niveau de l'itinéraire technique pour limiter l'impact négatif des légumineuses sur le rendement de la canne à sucre. Cependant, les services liés à la régulation des adventices et à l'enrichissement en azote exogène dépendent du développement des légumineuses. Par conséquent, il est essentiel de poursuivre les recherches, avec un focus particulier sur la date de semis et de destruction des légumineuses, afin de trouver un équilibre entre les services attendus et l'impact sur le rendement de la canne-à-sucre.

2. Intérêt des légumineuses pour la nutrition de la canne-à-sucre et la fertilité chimique du sol

***Hypothèse 5** : La quantité de N_2 fixé par la légumineuse dépend de sa capacité à croître. La proportion de N_2 fixé peut-être modifié par la gestion de l'itinéraire technique (fertilisation azotée et irrigation).*

Pour vérifier cette cinquième hypothèse, nous avons utilisé le dispositif expérimental, où nous avons suivi la fixation de l'azote atmosphérique par la légumineuse durant trois cycles de culture. Nos résultats confirment que la quantité d'azote N_2 fixée par la légumineuse est directement liée à sa croissance et est influencée par l'itinéraire technique, avec une diminution du pourcentage d'azote fixé en conditions fertilisé et pluvial (Chapitre 3).

***Hypothèse 6** : La décomposition rapide des résidus de légumineuse en cours de culture permet d'augmenter l'offre en azote pour la canne-à-sucre au cours du même cycle.*

Pour confirmer cette sixième hypothèse, nous avons utilisé plusieurs approches au sein de notre dispositif expérimental (Chapitre 3). La décomposition des résidus de légumineuse a été suivi au moyen d'une expérience utilisant des litter-bags. Nous avons également suivi la quantité d'azote minéral dans le sol pendant la décomposition de la légumineuse dans un traitement de référence (fertilisation azotée et irrigation). De plus, nous avons étudié le devenir de l'azote des légumineuses dans le système mulch-sol-plante en utilisant des légumineuses marquées au ^{15}N dans ce même traitement de référence.

Bien que les résidus de légumineuses se décomposent rapidement, la quantité d'azote effectivement restituée au sol sous forme minérale est relativement faible dans nos conditions expérimentales, probablement en raison de pertes importantes par volatilisation depuis le mulch de canne-à-sucre. Une partie de l'azote libéré lors de la décomposition des résidus de légumineuses est également immobilisée sur le mulch de canne-à-sucre. Il en résulte un faible apport des légumineuses à la nutrition de la canne-à-sucre au cours du même cycle. De plus, les pertes d'azote (issu de la légumineuse) du système étant équivalentes à la quantité d'azote apportée par la fixation, cela se traduit par un bilan nul en ce qui concerne la fertilité azoté (Chapitre 3).

***Hypothèse 7** : Une partie de l'azote issus de la décomposition de la légumineuse est intégré à la matière organique du sol et est restitué à la canne-à-sucre au cours des cycles suivants.*

Pour vérifier cette dernière hypothèse, nous avons suivi l'azote provenant des résidus de légumineuses marquées dans la canne-à-sucre pendant trois cycles de culture. Nos résultats confirment cette hypothèse, avec environ 7 % de l'azote des légumineuses récupéré chaque année par la canne-à-sucre. Cette quantité ne représente qu'une faible part (seulement 3 % en moyenne) de l'azote accumulée par la canne-à-sucre pendant sa croissance.

Les résultats rappelés ci-dessus suggèrent un intérêt limité des légumineuses pour la nutrition de la canne-à-sucre et la fertilité chimique du sol dans le contexte de La Réunion. Cependant ces résultats ne pourraient être extrapolé largement à d'autres conditions agro-

pédoclimatiques. Le faible apport d'azote exogène au système sol-plante par la légumineuse est dans notre cas lié à la fois au choix de favoriser la croissance de la canne au détriment de la légumineuse et à un cumul de conditions favorisant la volatilisation de l'azote. Par conséquent, il existe un potentiel d'amélioration significatif de la gestion de ce système.

Les données de la méta-analyse montrent une augmentation du rendement en canne-à-sucre dans environ un tiers des cas. Cela suggère que dans certains contextes, la facilitation est plus importante que la compétition. Le potentiel des légumineuses pour réduire la dépendance aux engrais azotés nécessite ainsi des efforts de recherche supplémentaires, et de mise au point technique, pour mieux définir les conditions dans lesquelles les légumineuses ont un effet positif sur l'alimentation azotée de la canne.

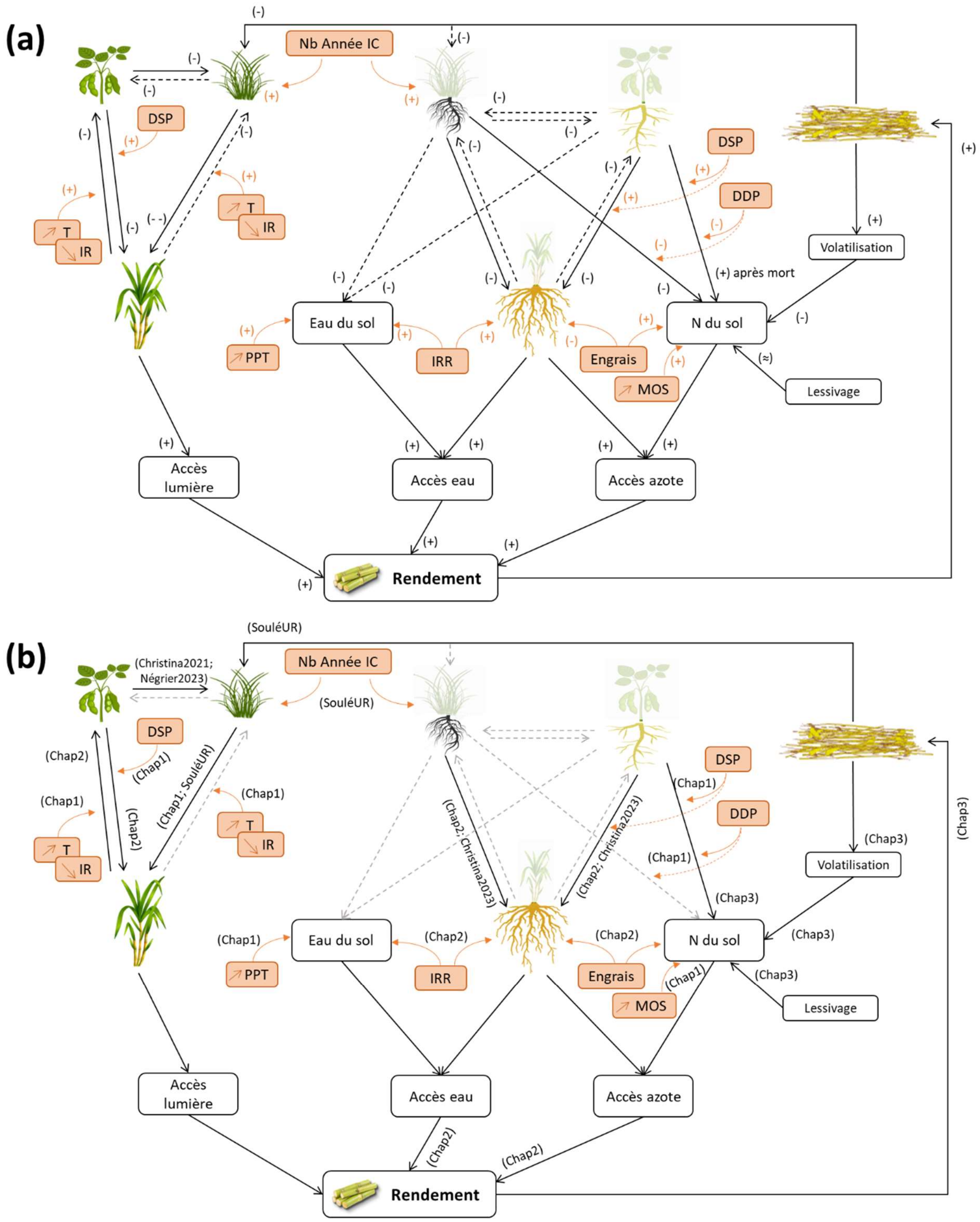

Figure 1. Schéma conceptuel des processus influençant le rendement de canne à sucre dans des systèmes en association canne x légumineuse x adventices. Le sens des effets positifs (+) ou négatifs (-) sont indiqués dans (a). Les flèches en plein représentent les effets démontrés dans les trois chapitres de thèse ou dans les articles en co-auteurs (Annexe). Les flèches en pointillées représentent les effets supposés mais non démontrés dans cette thèse. L'effet des leviers ou des facteurs pédoclimatiques sont représentés en orange (T : température, IR : largeur de l'interrang, DSP : date de semis précoce, DDP : date de destruction précoce, PPT : précipitation, IRR : irrigation, Engrais : fertilisation azotée, MOS : matière organique du sol, Nb Année IC : nombre d'année d'association). Les chapitres ou articles en annexe dont sont issus les informations sont indiquées en (b).

3. Perspectives scientifiques et opérationnelles

3.1. Réponse des traits racinaires de la canne-à-sucre à la compétition

Afin d'optimiser les systèmes de culture associant la canne-à-sucre et les légumineuses, il est nécessaire d'améliorer notre compréhension des mécanismes mis en jeu entre les racines de ces espèces. Il semble que le système racinaire de la canne à sucre adopte une stratégie d'évitement en réponse à la présence des légumineuses et/ou des adventices (Christina et al., 2023), ce qui a un impact négatif sur sa capacité à coloniser le sol. Les données sur la réponse du système racinaire de la canne-à-sucre recueillies au cours de cette thèse n'ont pas encore été complètement exploitées. Les travaux de thèse de Léa Chevalier (2022-2025), menés en collaboration entre le CIRAD et eRcane, permettront d'approfondir cette question à partir de ce jeu de données. Les premiers résultats indiquent un effet de compétition indirecte (réduction du volume de sol exploré) à travers une modification de la distribution racinaire et des traits racinaires de la canne-à-sucre, notamment une diminution de la longueur spécifique des racines dans certaines conditions de disponibilité en azote et en eau (Graillot, 2022).

Les travaux menés à La Réunion sont concentrés sur une variété de canne-à-sucre (R579) en association avec *Canavalia ensiformis* sur un site expérimental spécifique (Christina et al., 2023 ; Chevalier, 2021 ; Graillot, 2022). Les premières conclusions concernant la réponse du système racinaire de la canne-à-sucre devront être confrontées à d'autres conditions agro-environnementales, en particulier pour d'autres variétés de canne -à-sucre qui pourraient présenter des réponses différentes.

3.2. Abbatement des pertes d’azote lors de la décomposition des résidus de légumineuse

Les pertes significatives d'azote observées dans notre étude mettent en évidence la nécessité d'améliorer notre compréhension du cycle de l'azote dans ces systèmes. En particulier, il est essentiel d'approfondir nos connaissances sur les interactions entre le mulch de canne-à-sucre et les résidus de légumineuse, en accordant une attention particulière à l'impact présumé du mulch de canne-à-sucre sur les pertes par volatilisation.

Nous pouvons proposer plusieurs pistes à explorer et à évaluer en ce qui concerne la gestion de l'itinéraire technique afin de réduire ces pertes et d'optimiser les services fournis par les légumineuses dans ces systèmes. De nombreuses études ont montré que la volatilisation était limitée lorsque les résidus étaient enfouis. Par conséquent, l'approche la plus évidente consisterait à enfouir les résidus de légumineuses. Cependant, cette solution présente des inconvénients, notamment la perte des avantages associés au mulch (protection contre l'érosion, barrière à la levée des adventices, conservation de l'humidité du sol), et peut s'avérer difficile à mettre en œuvre pour les parcelles non mécanisables et à un stade avancé de croissance de la canne-à-sucre (environ 5 mois après la coupe). Néanmoins, la difficulté des méthodes réductrices en herbicide actuelles pour gérer les adventices sur le rang de canne (Mansuy et al., 2019 ; Chetty et al., 2023) pousse les recherches travailler sur des méthodes incluant la concentration du mulch de canne sur le rang de canne, ce qui pourrait permettre de limiter les pertes dans l'inter-rang.

En restant dans le système actuel d'un mulch en plein, plusieurs pistes peuvent être exploré pour limiter la volatilisation :

- Evaluer des espèces de légumineuse avec des ratios C:N plus élevés pour réduire la vitesse de minéralisation, ce qui permettrait de limiter les pertes par volatilisation.
- Favoriser l'infiltration de l'azote de la légumineuse nouvellement libéré dans le sol en optimisant l'irrigation (lame d'eau et intervalle d'application), où il sera moins susceptible d'être volatilisé.

3.3. Optimisation de l'itinéraire technique par modélisation

La gestion de ces associations nécessite d'être approfondi afin d'optimiser les bénéfices des légumineuses tout en limitant l'impact sur le rendement de la canne-à-sucre. Les récents progrès dans le développement de modèles de cultures pour les associations de culture offrent la perspective de pouvoir tester différentes stratégies dans des conditions pédoclimatiques variées, dont la mise en œuvre expérimentale serait complexe et coûteuse. Les résultats issus de cette thèse pourront servir de base pour le paramétrage d'un modèle de culture tel que STICS (Brisson et al., 2003). La méta-analyse a permis d'explorer un large éventail de conditions environnementales et agronomiques. Cependant, en raison de la multitude de facteurs par rapport au nombre d'études et à la variabilité des rendements mesurés, certains facteurs n'ont pas pu être testés expérimentalement et pourraient être testé avec un modèle de culture (tels qu'une fertilisation N additionnelle pour la légumineuse, la fertilisation P– en cours d'intégration dans STICS, la gestion des résidus, les densités de semis, des plantes de services avec des traits différents ou encore l'optimisation des dates de semis et de destruction). Les pistes évoquées précédemment pour limiter les pertes par volatilisation pourraient également être évaluées par cette approche.

3.4. Effet à long terme de ces systèmes sur la population d'adventices

La gestion des adventices se raisonne à la fois à l'échelle temporelle du cycle de culture mais aussi sur le long terme. De futurs travaux pourraient s'intéresser à l'évolution des communautés d'adventices et du stock semencier dans ces systèmes de culture. Dans la méta-analyse, nous avons émis l'hypothèse que l'impact sur le rendement en canne-à-sucre observée à long terme pouvait être lié à une augmentation de la pression des adventices au cours du temps (hypothèse confirmée par l'étude de Soulé et al., under review, Annexe). Une augmentation du stock semencier et de la densité des adventices peut être liée à : i) un changement de communauté, ii) une plus faible maîtrise des adventices par les légumineuses par rapport à un système conventionnelle augmentant ainsi le stock semencier, mais aussi iii) par un effet facilitateur induite par une augmentation de la disponibilité de l'azote (Sjursen et al., 2012). Ce dernier effet peut également modifier la composition de la flore adventices en favorisant les adventices nitrophile (Dayoub, 2017). Les études menées à La Réunion montrent que les légumineuses peinent à maîtriser les lianes et les grandes graminées tel que les fataques (*Megathyrsus maximus*, Mansuy et al., 2019). Ces dernières pourraient donc représenter un défi à moyen-long terme dans ces systèmes de culture. Des approches par modélisation pourraient permettre d'évaluer ces systèmes sur le long terme. La majorité des modèles de culture présentent toute fois l'inconvénient de ne pas représenter les communautés d'adventices. A notre connaissance, seul le modèle FlorSys permet actuellement de prédire la dynamique pluri-annuelle des adventices dans les systèmes de culture (Colbach et al., 2021).

3.5. Effet des légumineuses sur la fertilité physique, biologique et chimique du sol

Nous avons fait le choix de centrer cette thèse sur l'effet des légumineuses sur la fertilité azotée du sol. Cependant, les légumineuses peuvent également offrir d'autres services. Il s'agit, par exemple, de la prévention de l'érosion du sol par la plante vivante et ses résidus (Hartwig and Ammon, 2002 ; Scholberg et al., 2010), de l'accumulation de matière organique dans le sol qui assure une meilleure rétention en eau, un stockage du carbone et fournit un substrat pour la faune du sol (Scholberg et al., 2010) et l'amélioration de la structure du sol via leurs racines favorisant l'infiltration de l'eau (Scholberg et al., 2010 ; Sheaffer and Seguin, 2003). Pour ce dernier aspect, des travaux en cours menés en Argentine sur un sol très compacté, montrent que les légumineuses, en améliorant la structure du sol, favorisent une meilleure infiltration de l'eau et entraînent une augmentation du rendement en canne-à-sucre par rapport au témoin en monoculture (thèse de Luciana Martinez Calsina, Communication personnelle). Cet exemple illustre l'importance d'explorer les divers effets des légumineuses sur la fertilité globale du sol, qu'il s'agisse des aspects physiques, chimiques ou biologiques.

Les impacts d'une pratique culturale sur la fertilité du sol s'évaluent sur le long terme. Ainsi, il serait bénéfique de mener de futurs travaux sur une échelle de temps plus longue, éventuellement en utilisant des modèles de culture, afin de prendre en compte les cycles temporels sur lesquels se déroulent et se stabilisent ces processus.

4. Conclusion, vers une adoption des associations canne x légumineuses à La Réunion ?

La filière canne à sucre à la Réunion fait aujourd'hui face à plusieurs défis, en particulier, la nécessité de réduire l'utilisation des herbicides en lien avec les nouvelles réglementations, augmentation des coûts de productions et demandes sociétales. Face à ces défis, les instituts de recherche et le Réseau d'Innovation et de Transfert Agricole (RITA) ont étudié et promu différents itinéraires techniques innovants depuis une dizaine d'année. Parmi eux, l'utilisation de légumineuse en association dans les systèmes canniers en association a été largement mis en avant (Bourgaut, 2021) lors de nombreuses journées de transfert et de démonstration car elle permet notamment de réduire l'utilisation des herbicides de moitié (Mansuy et al., 2019). Pourtant force est de constater la faible adoption de cette pratique par les agriculteurs à La Réunion. L'adoption de l'association de culture dans le système cannier réunionnais fait face à de nombreuses barrières : l'impératif de production suffisante pour les usines sucrières limitant les pratiques induisant de légères baisses de rendement, le manque de disponibilité en semences de plantes de service en raison de l'absence de filière sur l'île, le manque de main d'œuvre alors que cette pratique demande une augmentation des interventions manuelles en particulier sur le rang de canne (Mansuy et al., 2019 ; Soulé et al., under review), ou encore l'absence de label permettant une meilleure valorisation économique de cette pratique. Un projet européen en cours sur les associations de culture (projet IntercropValues, incluant la canne à La Réunion comme cas d'étude) a pour objectif d'étudier les verrous et leviers au niveau de la chaîne de valeur pour identifier des solutions crédibles pouvant être adoptées par les agriculteurs et les acteurs impliqués sur l'ensemble de la chaîne, y compris les acteurs de la transformation des produits agricoles.

Cette thèse a toutefois permis de progresser dans la compréhension et quantifications des mécanismes d'intensification agroécologique actionnables via l'introduction de légumineuses dans la culture de canne à sucre. Certains de ces mécanismes pourraient être mieux valorisés si le cadre de contrainte réglementaire et économique évoluait en faveur d'une agriculture moins productiviste, moins impactante sur l'environnement et valorisant mieux le travail.

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Annexes

Supplementary Material. Chapitre 1

Appendix A: Complementary Material & Methods

Procedure for literature search and source paper selection

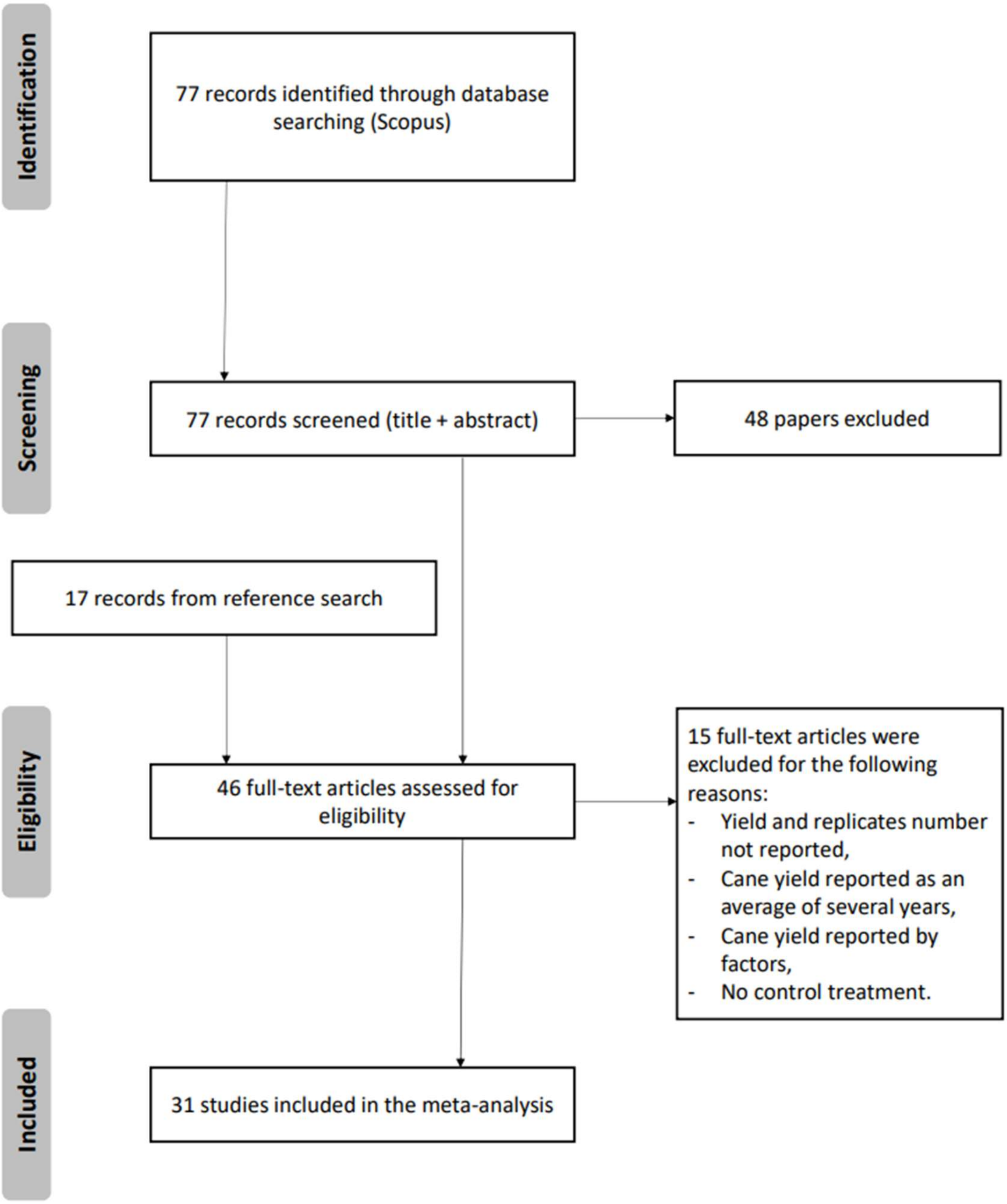

Figure A1. PRISMA diagram.

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Climate

Table A1. Characteristics of the types of climate

Köppen climate classification	Climate	n	Mean annual rainfall (mm)	Mean annual temperature (°C)	Altitude (m)
BWh	Desert	30	327	24,8	207
BSh	Semi-arid	25	699	24,9	354
Cfb	Oceanic	52	1722	21,9	1533
Cfa, Cwa	Subtropical	94	1421	21,7	173
Af, Aw, As	Tropical	114	1331	25,0	101

Selected studies for the meta-analysis

Table A2. Selected studies for the meta-analysis, from both data set: Reunion and Literature. Details on references are available in Appendix A.

Reference	Country	Duration (years)	Legume companion plant species	Soil texture	Mean annual precipitation (mm)	Mean annual temperature (°C)
Experiment 1	La Réunion (-20.903, 55.532)	2	*Vigna unguiculata*	Fine	1344	26.3
Experiment 2	La Réunion (-20.903, 55.532)	6	*Canavalia ensiformis, Crotalaria juncea, Crotalaria spectabilis, Desmodium intortum, V. unguiculata*	Fine	1344	26.3
Experiment 3	La Réunion (-20.903, 55.532)	3	*C. ensiformis*	Fine	1344	26.3
Experiment 4	La Réunion (-20.903, 55.532)	5	*C. ensiformis, D. intortum, V. unguiculata*	Fine	1344	26.3
Experiment 5	La Réunion (-20.903, 55.532)	1	*V. unguiculata*	Fine	1344	26.3
Experiment 6	La Réunion (-21.047, 55.682)	3	*C. ensiformis, V. unguiculata*	Fine	1603	22.6
Experiment 7	La Réunion (-21.067, 55.280)	1	*C. ensiformis, D. intortum*	Fine	816	20.1
de Souza Filho and de Andrade 1985	Brazil	3	*Phaseolus vulgaris*	Fine	1797	22
Parsons and Khubone 1999	South-Africa	1	*Arachis hypogaea, P. vulgaris*	Fine	1123	21.9
Gana and Busari 2001	Nigeria	2	*Glycine max, Sesbania rostrata, V. unguiculata,*	Coarse	1075	28.4
Nazir et al., 2002	Pakistan	1	*Lens culinaris, Pisum sativum*	Coarse	346	24.2
de Resende et al., 2003	Brazil	2	*C. ensiformis, C. juncea, C. spectabilis, Mucuna pruriens*	Coarse	1354	23.5
Parsons 2003	South-Africa	1	*G. max, P. vulgaris, V. unguiculata*	Fine	1123	21.9
Saini et al., 2003	India	3	*P. sativum*	Coarse	769	23.9
Berry et al., 2009	South-Africa	1	*A. hypogaea, M. pruriens, Phaseolus lunatus*	Coarse	861	20
Manimaran et al., 2009	India	1	*Vigna mungo*	Coarse	1048	28.7

Rasool et al., 2011	Pakistan	1	*Cicer arietinum, L. culinaris*	Coarse	941	21.3
Prellwitz and Coelho 2011	Brazil	1	*C. juncea*	Fine	1073	24.4
Ambrosano et al., 2013	Brazil	1	*Cajanus cajan, C. ensiformis, Crotolaria brevifolia, C. juncea, Crotolaria mucronata, M. pruriens*	Fine	1255	20.8
Rehman et al., 2014	Pakistan	2	*C. arietinum, G. max*	Medium	346	24.2
Teshome et al., 2015	Ethiopia	1	*G. max*	Fine	200	28.7
Kaur et al., 2015	India	2	*P. sativum*	Coarse	1363	22.5
Córdova-Gamas et al., 2016	Mexico	2	*C. cajan, C. ensiformis*	Fine	1646	26.3
Khippal et al., 2016a	India	2	*Cyamopsis tetragonoloba, V. mungo, Vigna radiata, V. unguiculata*	Medium	772	24.1
Khippal et al., 2016b	India	2	*C. arietinum, L. culinaris, P. sativum*	Medium	766	24.4
Nadiger al., 2017	India	1	*G. max, P. sativum*	Medium	602	25.2
Pereira et al., 2017	Brazil	1	*C. cajan*	Fine	1341	20
He et al., 2018	China	2	*V. radiata*	Medium	1363	22.5
She et al., 2019	China	2	*A. hypogaea*	Medium	1727	22.2
Yasin et al., 2018	Pakistan	1	*L. culinaris*	Medium	346	24.2
Zarekar et al., 2018	India	1	*A. hypogaea, V. radiata*	Medium	2674	26
Salazar Cajas 2019	Australia	2, 1	*G. max*	Medium	1457	19.8
Solanki et al., 2019	China	1	*A. hypogaea, G. max*	Medium	1363	22.5
Geetha et al., 2019	India	1	*C. juncea, G. max, L. culinaris, V. unguiculata*	Coarse	618	26.3
Jhansi 2020	India	1	*A. hypogaea, C. juncea, C. tetragonoloba, G. max, V. mungo, V. radiata, V. unguiculata*	Medium	1108	28.2
Tian et al., 2020 ; Luo et al., 2016	China	10	*G. max*	Medium	1727	22.2
Nadeem et al., 2020	Pakistan	2	*L. culinaris*	Coarse	346	24.2

Network trials in La Reunion: Trial's experimental design and crop management

Table A3. Details of Reunion trials. Experiment identification, sugarcane variety, year, crop cycle (P = plant crop, R = ratoon crop), beginning of the sugarcane crop cycle (planting or harvest date), end of the sugarcane crop cycle (harvest date), plot size, number of repetition and number of treatment (separated into control treatment = CT and intercrop treatment = IC)

Experiment	Variety	Year	Crop cycle	Planting or harvest date (beginning)	Harvest date (end)	Plot size (m^2)	Number of repetitions	Number of treatments
Experiment 1 (P12)	R587	2017-2018	P	01/10/2017	02/10/2018	234	2	1CT + 1IC
		2018-2019	R1	02/10/2018	18/09/2019	234	2	1CT + 1IC
Experiment 2 (P41)	R579	2014-2015	R3	08/04/2014	05/08/2015	60	4	1CT + 6IC
		2015-2016	R4	05/08/2015	02/08/2016	60	4	1CT + 6IC
		2016-2017	P	02/08/2016	24/07/2017	60	4	1CT + 4IC
		2017-2018	R1	24/07/2017	25/07/2018	60	4	1CT + 4IC
		2018-2019	R2	25/07/2018	30/07/2019	60	4	1CT + 4IC
		2019-2020	R3	30/07/2019	30/07/2020	60	4	1CT + 2IC
Experiment 3 (P25 Montagne)	R579	2017-2018	P	01/07/2017	03/07/2018	306	2	1CT + 1IC
		2018-2019	R1	03/07/2018	02/07/2019	306	2	1CT + 1IC
		2019-2020	R2	02/07/2019	07/07/2020	306	2	1CT + 1IC
Experiment 4 (P25 LEG)	R579	2011-2012	P	24/11/2011	20/11/2012	60	4	1CT + 7IC
		2012-2013	R1	20/11/2012	27/11/2013	60	4	1CT + 7IC
		2013-2014	R2	27/11/2013	09/09/2014	60	4	1CT + 7IC
		2014-2015	R3	09/09/2014	15/09/2015	60	4	1CT + 7IC
		2015-2016	R4	15/09/2015	13/09/2016	60	4	1CT + 8IC
Experiment 5 (P25 ITK)	R579	2012-2013	R1	20/11/2012	27/11/2013	60	4	1CT + 1IC
Experiment 6 (O.Fontaine)	R579	2015-2016	R4	23/07/2015	06/07/2016	60	3	1CT + 6IC
		2016-2017	P	13/10/2016	19/10/2017	675	3	1CT + 1IC
		2017-2018	R1	20/10/2017	23/10/2018	750	3	1CT + 1IC
Experiment 7 (A.Crescence)	R579	2014-2015	R1	23/10/2014	09/10/2015	60	3	1CT + 2IC

Table A4. Main soil characteristics in the 0-30 cm superficial soil layer. The soil type was indicated based on the WRB classification.

Experiment	Soil type	Year of analysis	pH	N (g/kg)	C (g/kg)	C/N	P (mg/kg)	K (cmol+/kg)	Ca (cmol+/kg)	Mg (cmol+/kg)	Na (cmol+/kg)	CEC (cmol+/kg)
Experiment 1	Nitisol	2017	6,56	1,34	15,86	11,83	44,96	0,81	4,98	3,15	0,18	9,21
Experiment 2	Nitisol	2013	6,21	2,01	24,17	12,01	88,27	1,00	6,64	3,04	0,10	8,81
Experiment 3	Nitisol	2017	6,06	2,01	22,03	10,94	95,65	0,66	7,19	2,89	0,14	11,67
Experiment 4	Nitisol	2017	6,06	2,01	22,03	10,94	95,65	0,66	7,19	2,89	0,14	11,67
Experiment 5	Nitisol	2017	6,06	2,01	22,03	10,94	95,65	0,66	7,19	2,89	0,14	11,67
Experiment 6	Nitisol	2013	5,08	2,38	30,45	12,79	73,35	0,10	1,23	0,38	0,05	2,57
Experiment 7	Andic cambisol	2016	4,40	2,34	30,54	13,08	81,68	0,04	0,11	0,07	0,06	1,95

Table A5. Fertilization rate and application date in the experiments. NA indicated missing data.

Experiment	Year	Date	Localisation	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
Experiment 1 (P12)	2017-2018	30/11/2017	Whole	75	50	120
		19/02/2018	Row	32	52	0
		19/02/2018	Row	0	54	0
		19/02/2018	Row	60	40	96
	2018-2019	23/10/2018	Row	120	80	192
		03/12/2018	Row	82,5	44	121
Experiment 2 (P41)	2015-2016	11/09/2015	Row	90	72	144
		03/11/2015	Row	45	36	72
		03/11/2015	Row	31,98	0	0
	2016-2017	26/08/2016	Row	67,5	54	108
		17/10/2016	Row	99,36	0	0
		17/10/2016	Row	0	58,32	0
	2017-2018	08/09/2017	Row	85	60	140
		20/10/2017	Row	32	12	40
		20/10/2017	Row	48	78	0
	2018-2019	27/08/2018	Row	105	84	168
		27/08/2018	Row	0	68,85	0
		16/10/2018	Row	61,18	0	0
	2019-2020	03/09/2019	Row	105	84	168
		03/09/2019	Row	0	67,5	0
		08/10/2019	Row	96,6	0	0
		08/10/2019	Row	0	0	102
Experiment 3 (P25 Montagne)	2017-2018	05/07/2017	Row	90	35	150
		13/10/2017	Row	23	0	0
		13/10/2017	Row	19,2	19,2	0
		13/10/2017	Row	0	0	51
	2018-2019	09/10/2018	Row	90	72	144
	2019-2020	30/08/2019	Row	126	49	210
		06/09/2019	Row	90	72	144
Experiment 4 (P25 LEG)	2011-2012	24/11/2011	Furrow	90	72	144
		08/02/2012	Row	15	12	24
	2012-2013	21/12/2012	Row	108	42	180
		19/02/2013	Row	46	0	0
	2013-2014	NA	Row	162	69	242
	2014-2015	01/10/2014	Row	72	28	120
		16/12/2014	Row	90	40,5	121,5
	2015-2016	07/10/2015	Row	100	45	135
		17/12/2015	Row	NA	NA	NA
Experiment 5 (P25 ITK)	2012-2013	21/12/2012	Row	108	42	180
		19/02/2013	Row	46	0	0
Experiment 6 (O.Fontaine)	2015-2016	NA	Row	162	63	270
	2016-2017	19/01/2017	Row	54	138	120
		23/01/2017	Row	0	48,6	0
	2017-2018	20/11/2017	Row	90	60	150
		11/01/2018	Row	65,7	167,9	219
Experiment 7 (A.Crescence)	2014-2015	12/11/2014	Row	130	58,5	175,5
		05/01/2015	Row	57	25,65	76,95

Table A6. Details on legume management in the sugarcane inter-row in the intercrop (IC) treatments. (Part 1)

Experiment	Year	IC modality	Sowing date	Legume species	Sowing density (based on 1.5m width)	Destruction (Y = yes ; N = no)
Experiment 1 (P12)	2017-2018	M2	22/02/2018	*Vigna unguiculata var. 40d*	12	N
	2018-2019	M2	19/11/2018	*Vigna unguiculata var. black*	22	N
Experiment 2 (P41)	2014-2015	M1	11/05/2014	*Desmodium intortum*	5,6	N
		M2	11/05/2014	*Canavalia ensiformis*	87	N
		M3	16/06/2014	*Desmodium intortum*	5,6	N
		M4	16/06/2014	*Canavalia ensiformis*	87	N
		M7	16/07/2014	*Desmodium intortum*	5,6	N
		M8	16/07/2014	*Canavalia ensiformis*	87	N
	2015-2016	M1	06/10/2015	*Desmodium intortum*	5,6	N
		M2	06/10/2015	*Canavalia ensiformis*	73	N
		M3	23/11/2015	*Desmodium intortum*	5,6	N
		M4	23/11/2015	*Canavalia ensiformis*	73	N
		M5	06/10/2015	*Canavalia ensiformis*	73	N
		M6	23/11/2015	*Canavalia ensiformis*	73	N
	2016-2017	M2	10/10/2016	*Canavalia ensiformis*	73	N
		M4	10/10/2016	*Vigna unguiculata var. 40d*	25	N
		M5	10/10/2016	*Crotalaria juncea*	20	N
		M6	10/10/2016	*Crotalaria spectabilis*	30	N
	2017-2018	M2	11/09/2017	*Canavalia ensiformis*	73	N
		M4	11/09/2017	*Vigna unguiculata var. 40d*	25	N
		M5	12/09/2017	*Crotalaria juncea*	20	N
		M6	11/09/2017	*Crotalaria spectabilis*	30	N
	2018-2019	M2	29/08/2018	*Canavalia ensiformis*	73	N
		M4	29/08/2018	*Vigna unguiculata var. black*	15	N
		M5	29/08/2018	*Crotalaria juncea*	24	N
		M6	29/08/2018	*Crotalaria spectabilis*	24	N
	2019-2020	M2	04/09/2019	*Canavalia ensiformis*	73	N
		M4	04/09/2019	*Vigna unguiculata var. black*	15	N
Experiment 3 (P25 Montagne)	2017-2018	M2	25/09/2017	*Canavalia ensiformis*	73	N
	2018-2019	M2	13/08/2018	*Canavalia ensiformis*	73	N
	2019-2020	M2	14/08/2019	*Canavalia ensiformis*	70	Y

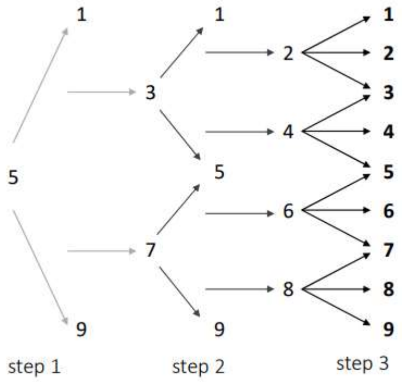
Table A6. Details on legume management in the sugarcane inter-row in the intercrop (IC) treatments. (Part 2)

Experiment	Year	IC modality	Sowing date	Legume species	Sowing density (based on 1.5m width)	Destruction (Y = yes ; N = no)
Experiment 4 (P25 LEG)	2011-2012	M1	30/01/2012	*Desmodium intortum*	5,4	Y
		M2	30/01/2012	*Vigna unguiculata var. rongaï*	15	Y
		M3	30/01/2012	*Canavalia ensiformis*	80	Y
		M6	30/01/2012	*Vigna unguiculata var. red*	14	Y
		M7	13/03/2012	*Canavalia ensiformis*	80	Y
		M8	13/03/2012	*Vigna unguiculata var. rongaï*	15	Y
	2012-2013	M1	20/11/2012	*Desmodium intortum*	2,4	Y
		M2	11/12/2012	*Vigna unguiculata*	15	Y
		M4	11/12/2012	*Canavalia ensiformis*	80	Y
		M6	13/01/2013	*Vigna unguiculata*	15	Y
		M7	13/01/2013	*Canavalia ensiformis*	80	Y
		M8	13/01/2013	*Vigna unguiculata*	15	Y
		M9	13/01/2013	*Canavalia ensiformis*	80	Y
	2013-2014	M1	12/12/2013	*Desmodium intortum*	5,6	N
		M2	12/12/2013	*Vigna unguiculata*	15	N
		M4	12/12/2013	*Canavalia ensiformis*	80	N
		M6	28/01/2014	*Vigna unguiculata*	15	N
		M7	28/01/2014	*Canavalia ensiformis*	80	N
		M8	28/01/2014	*Vigna unguiculata*	15	N
		M9	28/01/2014	*Canavalia ensiformis*	80	N
	2014-2015	M1	30/09/2014	*Desmodium intortum*	5,3	N
		M2	30/09/2014	*Vigna unguiculata var. red*	16	N
		M4	30/09/2014	*Canavalia ensiformis*	87	N
		M6	28/10/2014	*Vigna unguiculata var. red*	16	N
		M7	28/10/2014	*Canavalia ensiformis*	87	N
		M8	28/10/2014	*Vigna unguiculata var. red*	16	N
		M9	28/10/2014	*Canavalia ensiformis*	87	N
	2015-2016	M1	03/11/2015	*Vigna unguiculata var. red*	14	N
		M2	03/11/2015	*Vigna unguiculata var. red*	14	N
		M3	03/11/2015	*Vigna unguiculata var. red*	14	N
		M4	27/10/2015	*Vigna unguiculata var. 40d*	17	N
		M5	27/10/2015	*Vigna unguiculata var. 40d*	17	N
		M6	26/10/2015	*Canavalia ensiformis*	58	N
		M7	26/10/2015	*Canavalia ensiformis*	58	N
		M8	26/10/2015	*Canavalia ensiformis*	58	N
Experiment 5 (P25 ITK)	2012-2013	M5	15/12/2012	*Vigna unguiculata var. red*	14	N
Experiment 6 (O.Fontaine)	2015-2016	M1	06/09/2015	*Vigna unguiculata var. red*	14	Y
		M2	06/09/2015	*Vigna unguiculata var. red*	14	N
		M3	06/09/2015	*Vigna unguiculata var. 40d*	17	Y
		M4	06/09/2015	*Vigna unguiculata var. 40d*	17	N
		M5	06/09/2015	*Canavalia ensiformis*	58	N
		M6	06/09/2015	*Canavalia ensiformis*	58	Y
	2016-2017	M1	12/01/2017	*Canavalia ensiformis*	73	N
	2017-2018	M1	12/12/2017	*Canavalia ensiformis*	73	N
Experiment 7 (A.Crescence)	2014-2015	M2	23/10/2014	*Canavalia ensiformis*	80	N
		M4	02/12/2014	*Canavalia ensiformis*	80	N

Notation methods used to assess ground cover by plants

Table A7. Notation method used to assess ground cover by plants (companion plants and weeds). The choice of notation was made by following a multi-step decision tree. We estimate it to be above or below a value (e.g., less than 50% cover in the first step, at least one individual per m^2 in the second step, etc.).

Notation	Equivalence in percentage	Coverage
1	1	Species present but rare
2	7	Less than one individual per m^2
3	15	at least one individual per m^2
4	30	30% of coverage
5	50	50% of coverage
6	70	70% of coverage
7	85	High coverage
8	93	Very little apparent soil
9	100	Full coverage



Species' growth habits retained

Table A8. Species' growth habits. The legume species were classified according to their usual growth habit (creeping, erected, semi-erected, semi-twining, and twining) as identified by the Useful Tropical Plants (https://tropical.theferns.info/) and the PROTA4U (https://www.prota4u.org/) databases.

Legume species	Growth habit retained
Arachis hypogaea	Creeping
Cajanus cajan	Erected
Canavalia ensiformis	Semi-twining
Cicer arietinum	Semi-erected
Crotalaria juncea	Erected
Crotolaria spectabilis	Erected
Cyamopsis tetragonoloba	Erected
Desmodium intortum	Creeping
Glycine max	Semi-erected
Lens culinaris	Semi-erected
Mucuna pruriens	Twining
Phaseolus lunatus	Twining
Phaseolus vulgaris	Semi-twining
Pisum sativum	Semi-twining
Sesbania rostrata	Erected
Vigna mungo	Semi-erected
Vigna radiata	Semi-erected
Vigna unguiculata	Semi-twining

Coefficient of variation

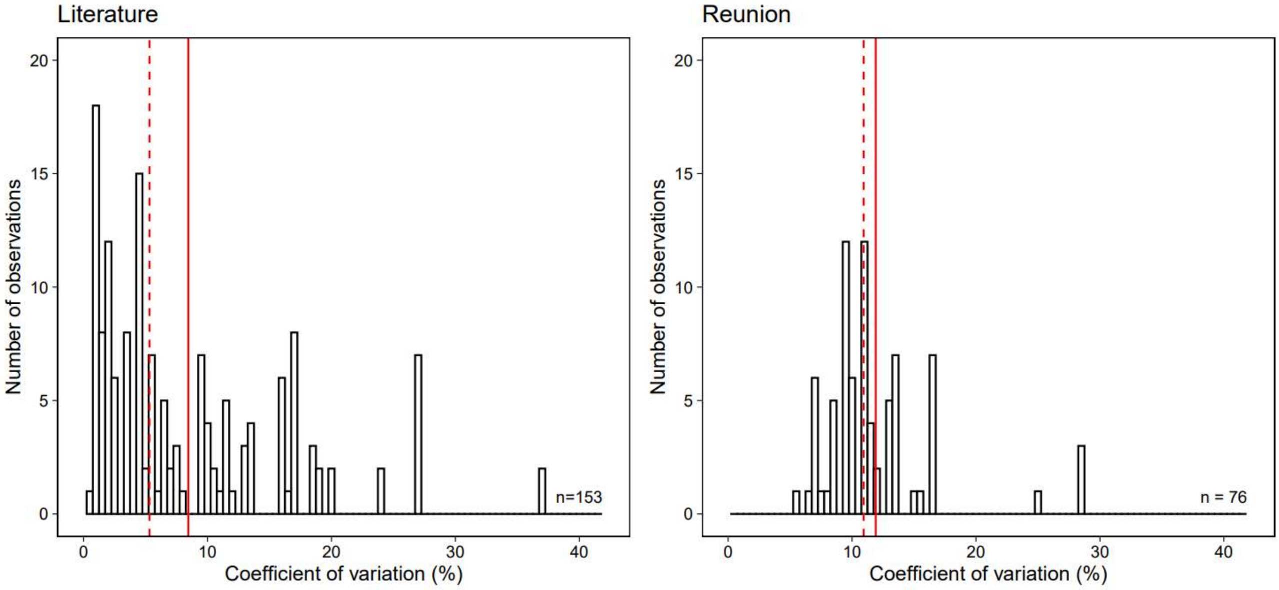


Figure A2. Histogram of coefficients of variation of sugarcane yield in the literature and the Reunion dataset. The solid and dashed lines correspond to the mean and median values respectively.

Preliminary analysis

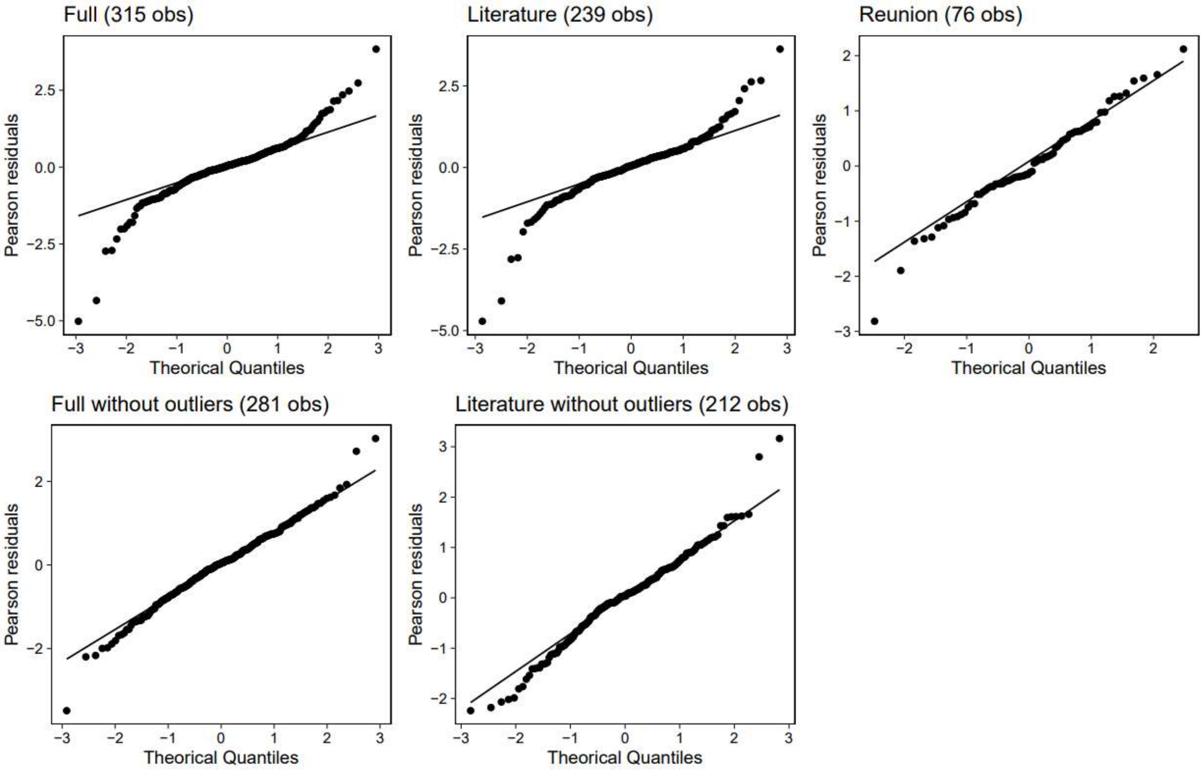


Figure A3. Normal QQ plot before and after outliers' removal for the three datasets. (Model beyond the optimal)

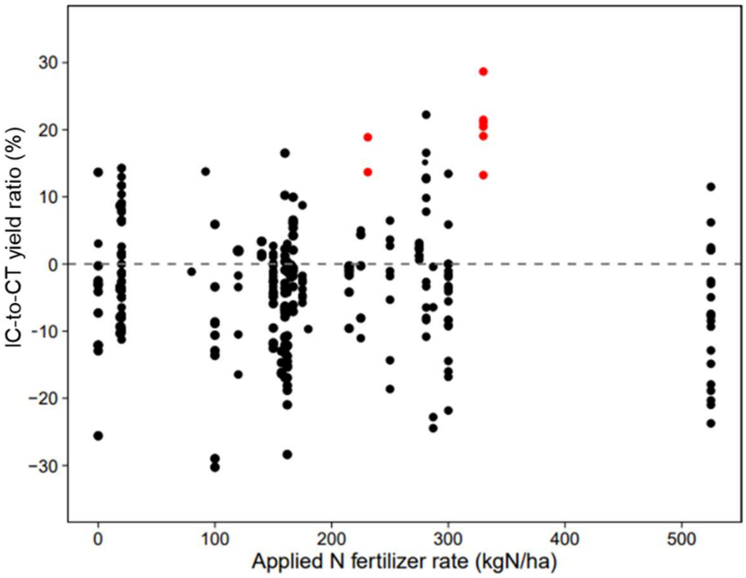


Figure A4. Effect of IC-to-CT sugarcane yield ratio as a function of applied N fertilizer rate (kg N/ha) in the full dataset (n=281). The red dots correspond to the observations from He et al. 2018 . The indicator variable "He2018" was a significant covariate for the Model 1 (F=9.02, P < 0.005) and the Model 2 (F= 9.27, P < 0.05).

Residuals diagnostics of the three models

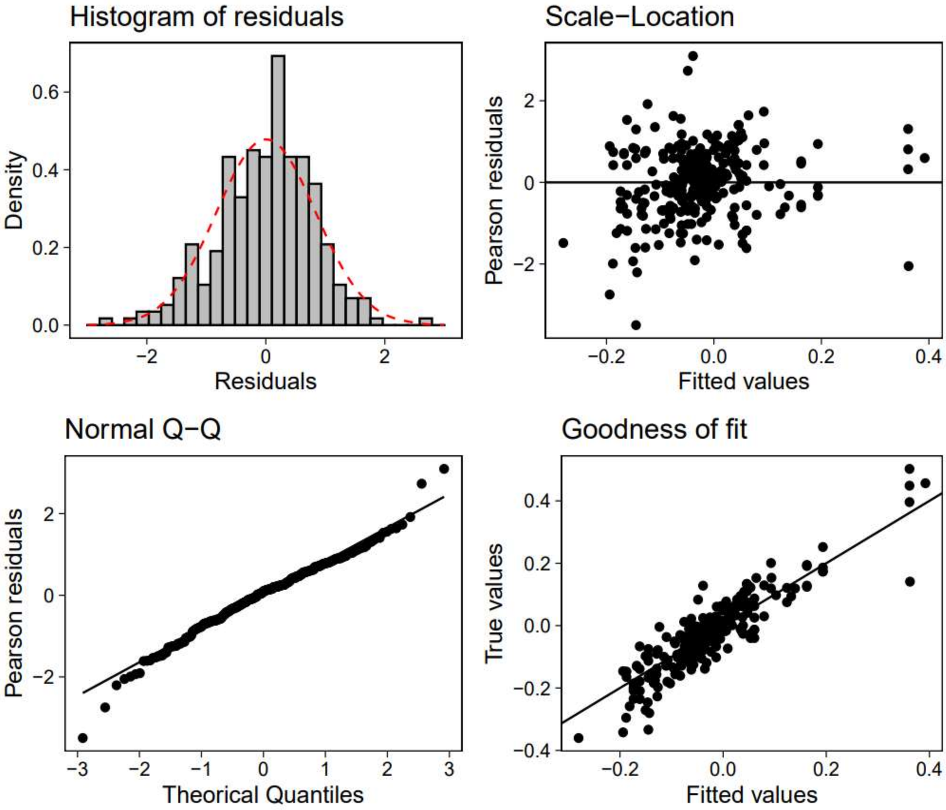


Figure A5. Residuals diagnostics of the Model 1

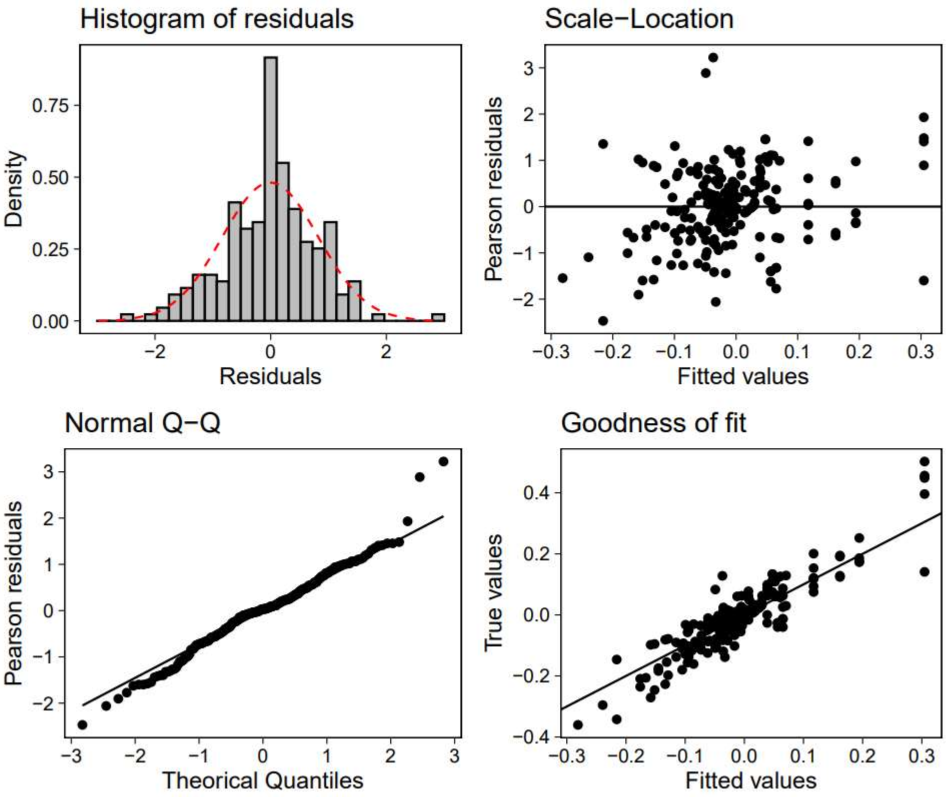


Figure A6. Residuals diagnostics of the Model 2

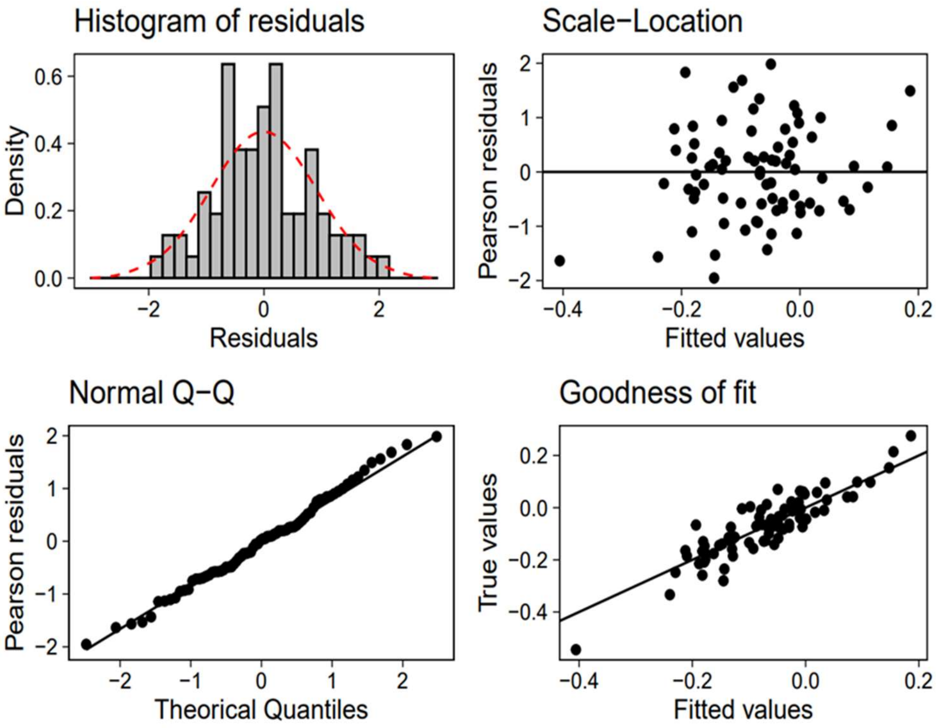


Figure A7. Residuals diagnostics of the Model 3

Publication bias

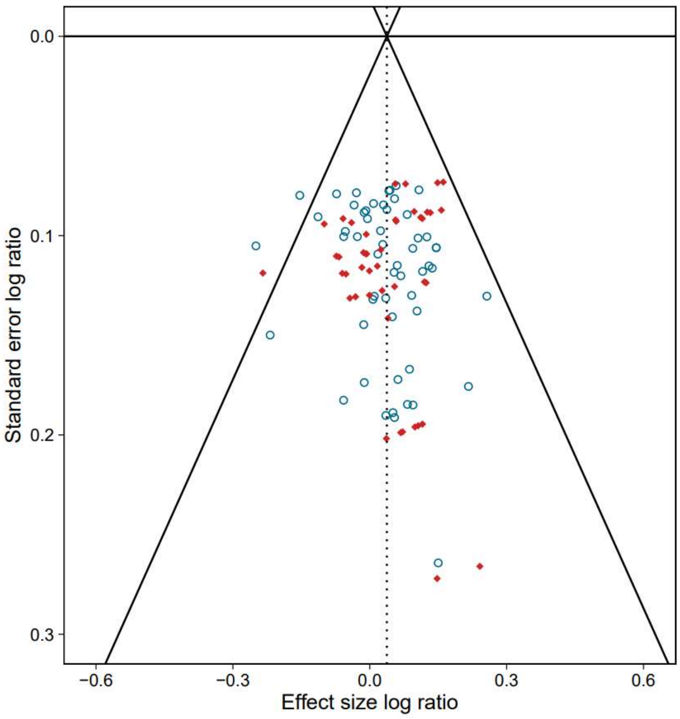


Figure A8. Funnel plot on the marginal deviations from the random-effects model added to the mean logarithmic yield ratio of the dataset (vertical dotted line). The diagonal lines represent the 95% CI limits around the effect size log ratio. Model 1 was applied to the data with a CV > 5% and without outliers identified by Cook's distances. Blue circles represent observations from the literature dataset (n=55), and red filled points represent observations from the Reunion dataset (n=44). Skewness is -0.03, P=0.67 (all observations), 0.10, P=0.27 (literature observations) and −0.12, P=0.26 (Reunion observations).

Appendix B: Complementary Results

Sugarcane yield response to intercrop treatment – Data without outliers

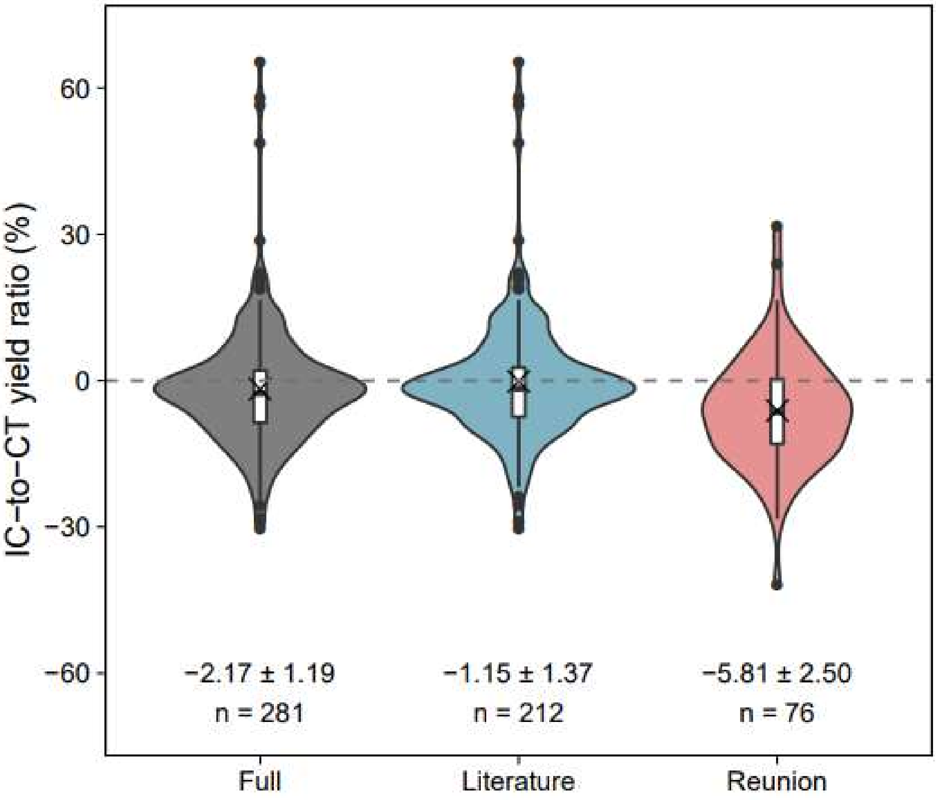


Figure B1. The IC-to-CT yield ratio depending on the data sets with outliers detected with Cook's distance removed. The horizontal lines within boxes indicate medians and the crosses indicated the mean. The upper and lower edges of boxes indicate 75th and 25th percentiles, whiskers below and above the boxes indicate the 10th and 90th percentiles. The mean value, confidence intervals (±) and the number of observations (n) are indicated below the box plots.

Results of the random-effects models developed to determine the influence of explanatory covariates on the IC-to-CT yield ratio

Table B1. Results of the Model 1 - Full dataset (n=281)

Model 1 - Full dataset (n=281)			
AIC	-500.0626		
AIC null model	-597.1699		
Marginal R^2	0.4289785		
Conditional R^2	0.7377387		
Random variables	**Estimate**		
Trial_Control	0.0634		
Residuals	0.0584		
Fixed variables	**df**	**F**	**P**
Climate	4	6.236319	0.0002
Mean annual temperature	1	3.450911	0.065
Climate x Mean annual temperature	4	6.590614	0.0001
Altitude	1	3.495948	0.0633
Growth habit	4	2.127128	0.0798
Planding day of CC	1	3.262063	0.0728
Width of the interrow	1	3.105049	0.0799
Number of years of IC	1	3.08111	0.0823
He2018	1	9.017352	0.0034

Table B2. Results of the Model 2 - Full dataset (n=212)

Model 2 - Literature dataset (n=212)			
AIC	-375.3915		
AIC null model	-439.425		
Marginal R^2	0.3564603		
Conditional R^2	0.7651307		
Random variables	**Estimate**		
Trial_Control	0.07323042		
Residuals	0.05551599		
Fixed variables	**df**	**F**	**P**
Climate	4	3.952	0.0057
Mean annual temperature	1	18.12177	0.0001
Soil organic carbon	1	2.242314	0.1383
Soil texture	2	2.661654	0.0762
Planding day of CC	1	2.602997	0.1093
Crop cycle	1	3.08143	0.0831
Number of years of IC	1	2.265133	0.1364
He2018	1	9.266626	0.0032

Table B3. Results of the Model 3 - Full dataset (n=76)

Model 3 - Reunion dataset (n=76)			
AIC	-117.4266		
AIC null model	-130.9951		
Marginal R^2	0.2848679		
Conditional R^2	0.7746869		
Random variables	**Estimate**		
Trial_Control	0.09589755		
Residuals	0.06504036		
Fixed variables	**df**	**F**	**P**
Soil organic carbon	1	8.881934	0.0074
Destruction day of CC	1	7.871815	0.0071
Weeds mean ground cover	1	7.194264	0.0098
Legume mean ground cover	1	5.713845	0.0206

Climate and soil effects

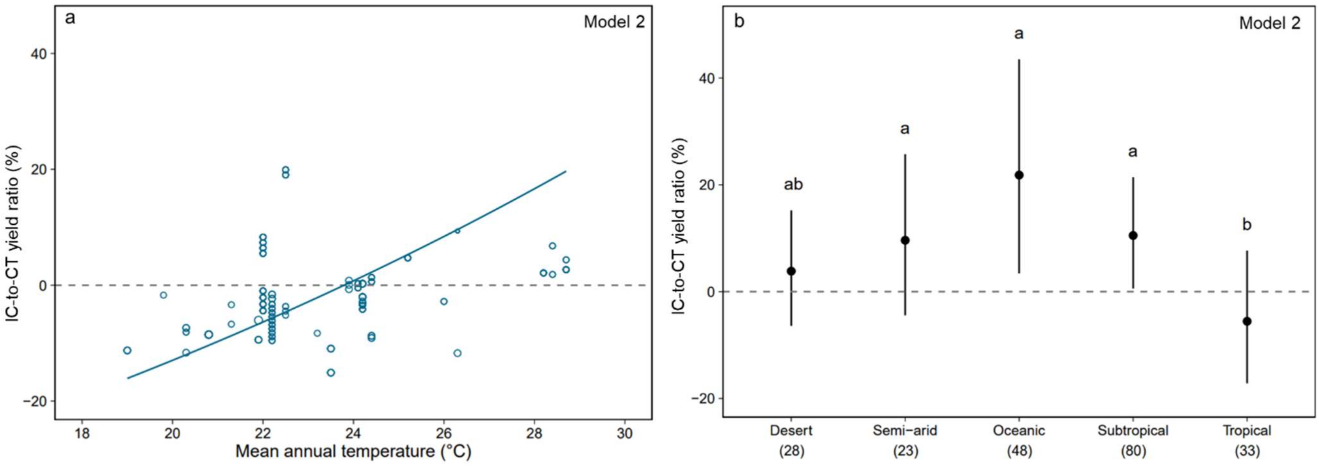


Figure B2. The IC-to-CT yield ratio depending on (a) the mean annual temperature (°C) and (b) the type of climate in the literature data set (Model 2). (a) The line represents the fitted random-effects model regression. The open circles represent the literature predicted values. (b) Values are weighted mean effect sizes, and error bars show the 95% confidence interval (CI). The mean effect size is considered significant if the 95% CI does not include 0. Treatments with the same letter were not significantly different.

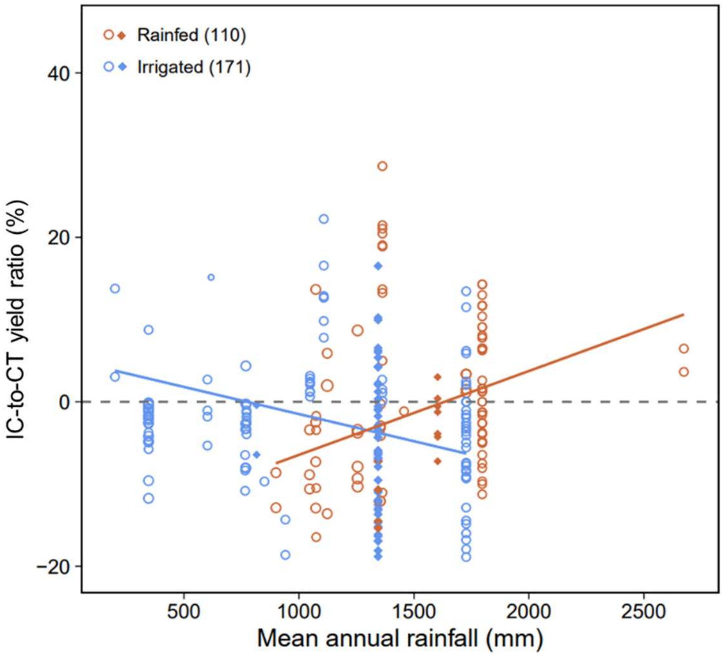


Figure B3. The IC-to-CT yield ratio depending on mean annual rainfall in rainfed and irrigated trials. Lines represent the linear regression. The open circles and filled points represent the observed values in the literature and Reunion datasets, respectively.

Management effects

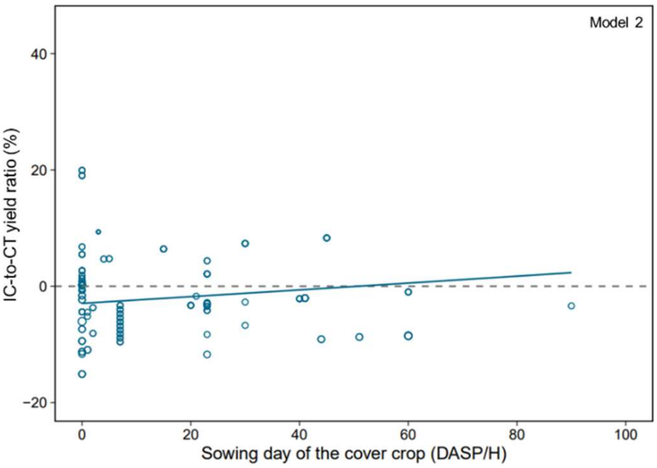


Figure B4. The IC-to-CT yield ratio depending on the legume sowing date (in days after planting or harvest DASP/H) in the literature data set (Model 2). The line represents the fitted random-effects model regression. The open circles represent the literature predicted values.

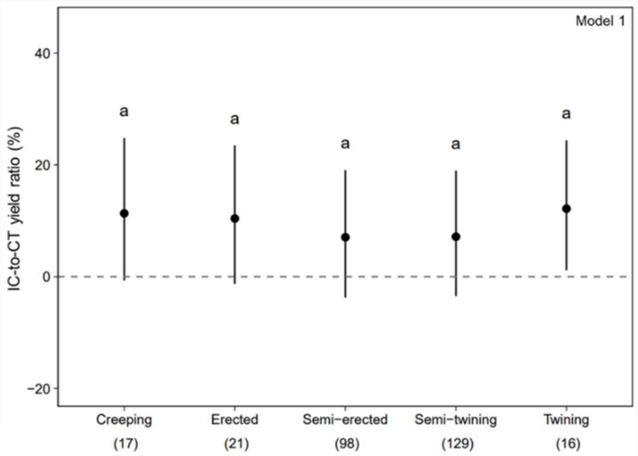


Figure B5. The IC-to-CT yield ratio depending on legumes growth habits in the full dataset (Model 1). Values are weighted mean effect sizes, and error bars show the 95% confidence interval (CI). The mean effect size is considered significant if the 95% CI does not include 0. Treatments with the same letter were not significantly different.

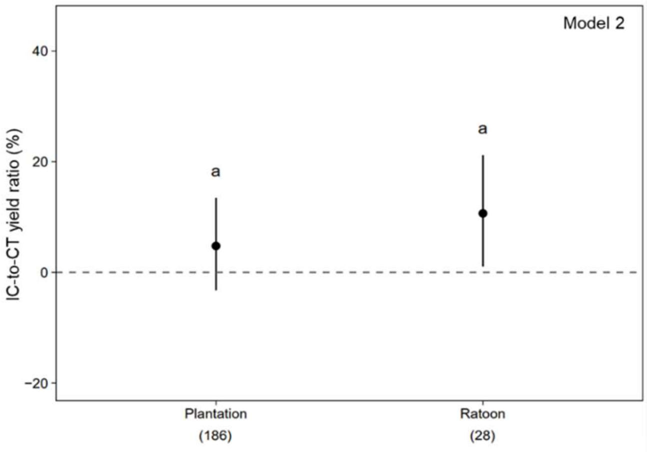


Figure B6. The IC-to-CT yield ratio depending on the type of sugarcane cycle (plantation vs. ratoon crop) in the literature dataset (Model 2). Values are weighted mean effect sizes, and error bars show the 95% confidence interval (CI). The mean effect size is considered significant if the 95% CI does not include 0. Treatments with the same letter were not significantly different.

Global mean ground cover (Legumes and weeds) effects on sugarcane yield

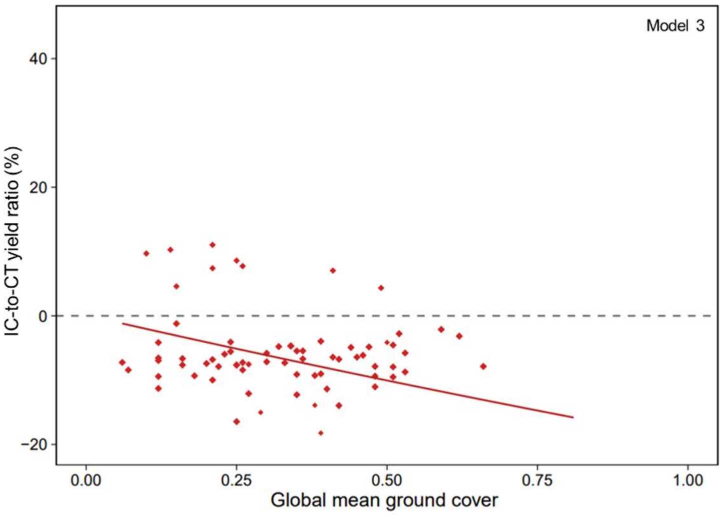


Figure B7. The IC-to-CT yield ratio depending on the global mean ground cover in the Reunion dataset (Model 3). The line represents the fitted random-effects model regression. The filled points represent the predicted values by the model in the Reunion dataset.

Effect of intercropping with legume over the years (Restricted dataset)

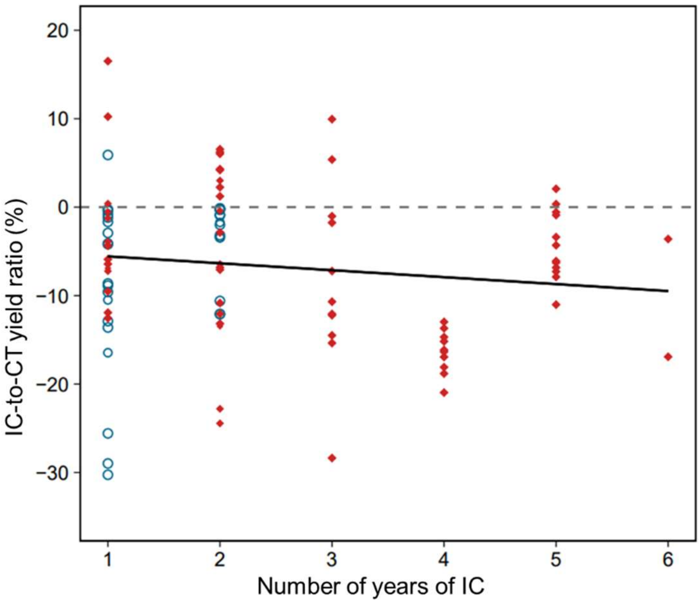


Figure B8. The IC-to-CT yield ratio depending on the number of years since the implementation of the intercropping treatment (years) in a restricted dataset with only the trials that did not replant each year (n= 104). The Lines represent the linear regression. The blue open circles and red filled points represent the observed values in the literature and Reunion datasets, respectively.

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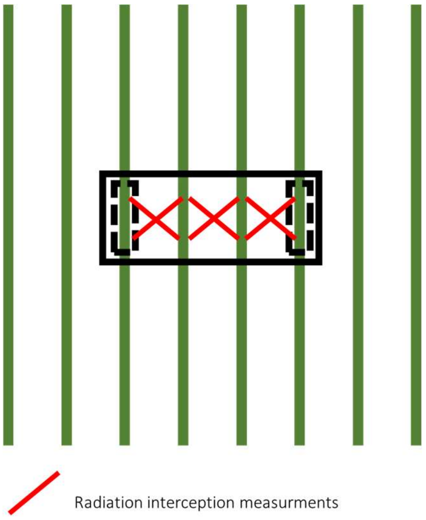

Figure S1. Radiation interception measurements with the sunscan probe.

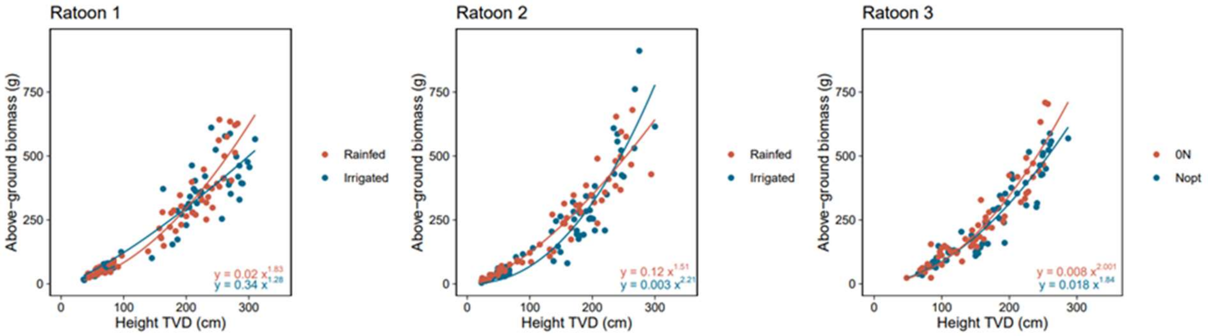

Figure S2. Relationship between sugarcane aboveground dry mass (g) and height TVD (cm) using global models for Ratoon 1, 2, and 3. Allometric relationships are represented by lines; measurements are represented by points.

Table S1. Allometric relationships selected to estimate aboveground dry mass (ADM, g) for TVD height (H, cm) in the inventory plots.

Cycle	Treatment	Equation	rRMSE
Ratoon 1	W	$ADM = 0.3368 * H^{1.2805}$	25.20
	0W	$ADM = 0.01814 * H^{1.83112}$	
Ratoon 2	W	$ADM = 0.002564 * H^{2.213214}$	30.60
	0W	$ADM = 0.1201 * H^{1.5051}$	
Ratoon 3	Nopt	$ADM = 0.01788 * H^{1.84514}$	21.97
	0N	$ADM = 0.008577 * H^{2.001236}$	

Table S2. Dry mass and N mass values used for the nitrogen recovery efficiency calculations (2.5.4.)

Compartment		**3 MAH**		**5 MAH**		**7 MAH**		**Harvest (12 MAH)**	
		Dry mass (t/ha)	**N mass (kg/ha)**	**Dry mass (t/ha)**	**N mass (kg/ha)**	**Dry mass (t/ha)**	**N mass (kg/ha)**	**Dry mass (t/ha)**	**N mass (kg/ha)**
SC		7,5	93,6	32,5	215,6	60,0	381,4	46,0	175,1
CP		0,2	7,4	0,6	23,6	0,3	12,1		
Mulch		9,5	49,4	8,4	76,5	9,7	70,9	7,0	56,3
Soil	0-5cm	493,0	908,0	493,0	908,0	493,0	908,0	493,0	908,0
	5-10cm	493,0	888,1	493,0	888,1	493,0	888,1	493,0	888,1
	10-20cm	986,0	1718,3	986,0	1718,3	986,0	1718,3	986,0	1718,3
	20-30cm	986,0	1483,8	986,0	1483,8	986,0	1483,8	986,0	1483,8

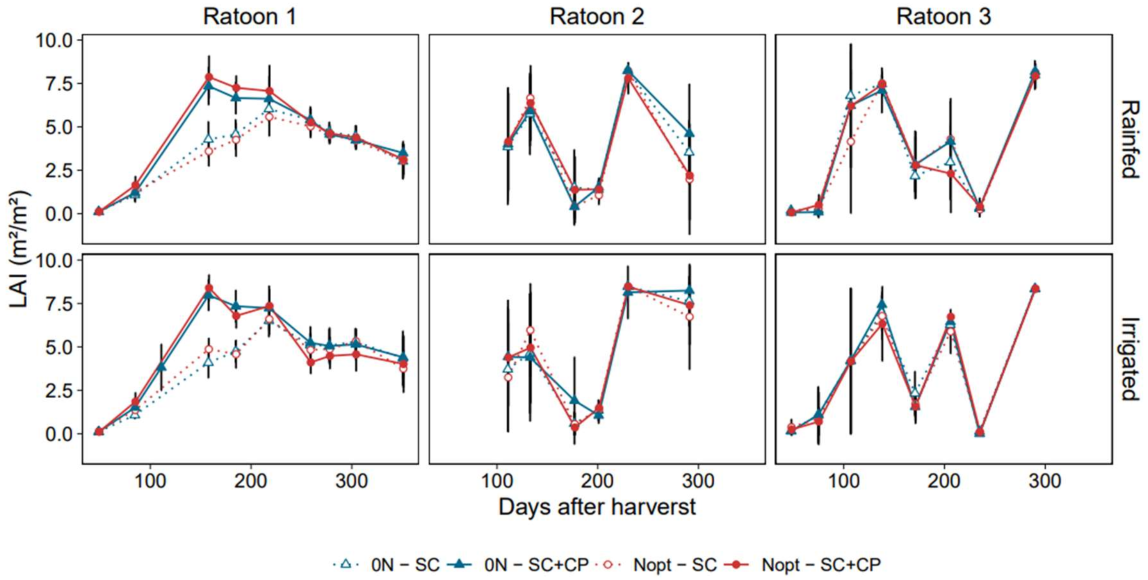

Figure S3. Dynamics of the LAI of sugarcane and CP in response to W (Rainfed or irrigated), N (0N or Nopt), and CS (SC or SC+CP) treatments, and their interaction for the three ratoon cycles (Ratoon 1: 2019-2020, Ratoon 2: 2020-2021, Ratoon 3: 2021-2022). Dots represent the mean, and bars represent the standard deviation.

Table S3. Structural equation model (SEM), showing direct and indirect effects of companion plant biomass and sugarcane parameters on (a) sugarcane yield (Yield_{SC}) and (b) lodging (Lodging_{SC}). The explanatory variables were the companion plant biomass (ADM_{CP}), sugarcane aboveground dry mass (ADM_{SC}) and root biomass (Root_DM_{SC}), height (Height_{SC}), numbers of tillers (Nb_Till_{SC}), and lodging (Lodging_{SC} for (a)). All the explanatory variables were measured at 5 MAH, except for lodging (7 MAH).

Model a - ADM_{SC} (n=96)			
χ^2	11,68		
pvalue	0,069		
Random variables			
Comparative Fit Index	0,982		
Tucker-lewis Index	0,955		
RMSEA	0,982		
Standardized Root Mean Square Residual	0,982		
Response variables	**Explanatory variables**	**Estimate**	**P**
Yield_{SC}	ADM_{SC}	0,28	0,010
	Lodging_{SC}	-0,41	0,000
	Heigth_{SC}	0,69	0,000
ADM_{SC}	Nb_Till_{SC}	0,57	0,000
	Height_{SC}	0,61	0,000
Lodging_{SC}	ADM_{SC}	0,70	0,000
	ADM_{CP}	0,24	0,003
Nb_Till_{SC}	ADM_{CP}	-0,34	0,000
Height_{SC}	ADM_{SC}	-0,24	0,016

Model b - Lodging_{SC} (n=48)			
χ^2	8,27		
pvalue	0,082		
Random variables			
Comparative Fit Index	0,940		
Tucker-lewis Index	0,851		
RMSEA	0,149		
Standardized Root Mean Square Residual	0,065		
Residuals	0,056		
Response variables	**Explanatory variables**	**Estimate**	**P**
ADM_{CP}	Nb_Till_{SC}	0,70	0,000
Lodging_{SC}	ADM_{SC}	0,34	0,004
	Root_DM_{SC}	-0,37	0,025
Root_DM_{SC}	Nb_Till_{SC}	0,36	0,016
	ADM_{SC}	-0,66	0,000
ADM_{SC}	ADM_{SC}	-0,26	0,087

Table S4. Details in nitrogen derived from fertilizer (NdfF) and nitrogen recovery efficiency at each sampling date for the first application ^{15}N urea date. MAH: month after harvest, SC: sugarcane, CP: companion plant (*C. ensiformis*).

Treatment	Compartment		3 MAH		5 MAH		7 MAH		Harvest (12 MAH)	
			NdfF (%)	NRE (%)	NdfF (%)	NRE (%)	NdfF (%)	NRE (%)	NdfF (%)	NRE (%)
SC	SC	center	30,65	41,89	13,73	43,22	10,55	58,76	2,75	7,04
		adjacent	0,03	0,04	0,08	0,26	2,62	14,59	0,28	0,72
	Mulch		12,22	8,93	7,50	8,36	11,64	12,02	6,15	4,71
	Soil	0-5cm	1,70	22,59	1,73	22,91	1,87	24,75	1,53	20,28
		5-10cm	0,55	7,07	0,24	3,11	0,81	10,49	0,20	2,60
		10-20cm	0,32	7,94	0,08	1,93	0,12	2,90	0,15	3,78
		20-30cm	0,03	0,63	0,07	1,49	0,12	2,63	0,08	1,79
SC + CP	SC	Int	30,52	37,63	12,57	37,86	11,31	58,61	4,73	9,73
		Ext	0,07	0,09	0,09	0,26	0,09	0,44	0,27	0,55
	CP	center	17,14	1,94	5,54	1,69	3,04	0,74		
		adjacent	0,05	0,01	0,20	0,08	0,07	0,01		
	Mulch		13,08	7,20	7,50	7,63	10,70	11,10	6,42	6,34
	Soil	0-5cm	1,33	17,60	0,97	12,82	2,03	26,88	1,59	21,11
		5-10cm	0,84	10,84	0,26	3,42	0,57	7,43	0,64	8,36
		10-20cm	0,13	3,35	0,11	2,75	0,27	6,69	0,12	3,09
		20-30cm	0,04	0,80	0,08	1,70	0,13	2,76	0,10	2,25

Table S5. Details in nitrogen derived from fertilizer (NdfF) and nitrogen recovery efficiency at each sampling date for the second application ^{15}N urea date. MAH: month after harvest, SC: sugarcane, CP: companion plant (*C. ensiformis*).

Treatment	Compartment		5 MAH		7 MAH		Harvest (12 MAH)	
			NdfF (%)	NRE (%)	NdfF (%)	NRE (%)	NdfF (%)	NRE (%)
SC	SC	center	18,85	59,35	13,45	57,33	5,85	14,95
		adjacent	0,16	0,50	0,36	1,98	0,08	0,20
	Mulch		13,36	14,22	10,65	11,09	9,87	7,82
	Soil	0-5cm	0,87	11,56	0,76	10,13	1,34	17,79
		5-10cm	0,26	3,32	0,35	4,53	0,28	3,57
		10-20cm	0,12	3,13	0,11	2,79	0,14	3,55
		20-30cm	0,09	2,02	0,09	1,85	0,09	1,99
SC + CP	SC	Int	17,54	52,86	12,71	65,84	3,35	6,90
		Ext	0,45	1,35	0,10	0,53	0,15	0,30
	CP	center	10,75	3,47	6,30	0,82		
		adjacent	0,60	0,22	0,01	0,00		
	Mulch		11,99	11,48	7,31	10,40	4,54	4,52
	Soil	0-5cm	0,90	11,90	1,29	17,16	1,53	20,26
		5-10cm	0,39	5,09	0,36	4,68	0,42	5,48
		10-20cm	0,06	1,40	0,10	2,62	0,24	6,14
		20-30cm	0,10	2,17	0,15	3,14	0,11	2,42

Supplementary Material. Chapitre 3

Table S1. Nitrogen derived from legume residues (NdF), and legume nitrogen recovery efficiency in the sugarcane (SC), mulch, and soil layers in the ^{15}N labelling experiments.

Experiment 1 (2020-2022) - Application in June 2020

Compartment		Ratoon 1 (2019-2020)				Ratoon 2 (2020-2021)				Ratoon 3 (2021-2022)			
		July 2020		October 2020		March 2021		October 2021		March 2022		October 2022	
		NdfF (%)	NRE (%)	NdfF (%)	NRE (%)	NdfF (%)	NRE (%)	NdfF (%)	NRE (%)	NdfF (%)	NRE (%)	NdfF (%)	NRE (%)
SC		0,2 ± 0,1	1,8 ± 0,7	2,4 ± 0,8	7,7 ± 2,5	3,4 ± 0,8	12,7 ± 5,4	2,2 ± 0,5	5,5 ± 1,3	8,5 ± 5,5	31,7 ± 20,5	1,1 ± 0,2	7,4 ± 2,1
Mulch		65,1 ± 31,3	41,2 ± 31,6	50,4 ± 5,6	34,6 ± 5,2	nd	nd	nd	nd	nd	nd	nd	nd
Soil	0-5cm	0,4 ± 0,3	7,1 ± 6,0	1,2 ± 0,1	22,3 ± 2,7	nd	nd	nd	nd	nd	nd	nd	nd
	5-10cm	0,2 ± 0,3	3,9 ± 5,1	0,1 ± 0,1	2,9 ± 1,2	nd	nd	nd	nd	nd	nd	nd	nd
	10-20cm	0,0 ± 0,1	0,6 ± 3,4	0,1 ± 0,06	3,7 ± 2,4	nd	nd	nd	nd	nd	nd	nd	nd
	20-30cm	0,0 ± 0,0	1,8 ± 1,6	0,0 ± 0,0	0,0 ± 1,3	nd	nd	nd	nd	nd	nd	nd	nd

Experiment 2 (2021-2022) - Application in June 2021

Compartment		Ratoon 2 (2020-2021)				Ratoon 3 (2021-2022)			
		July 2021		October 2021		March 2022		October 2022	
		NdfF (%)	NRE (%)	NdfF (%)	NRE (%)	NdfF (%)	NRE (%)	NdfF (%)	NRE (%)
SC		0,3 ± 0,2	0,7 ± 0,5	3,8 ± 0,4	6,6 ± 0,7	0,9 ± 1,3	1,6 ± 2,3	1,5 ± 0,1	5,4 ± 0,3
Mulch		30,9 ± 1,7	21,4 ± 4,7	28,8 ± 5,8	11,2 ± 0,5	nd	nd	nd	nd
Soil	0-5cm	1,3 ± 0,4	17,2 ± 3,8	2,4 ± 1,1	27,5 ± 12,7	nd	nd	nd	nd
	5-10cm	0,7 ± 0,2	9,0 ± 0,9	0,2 ± 0,1	2,5 ± 1,2	nd	nd	nd	nd
	10-20cm	0,2 ± 0,1	2,6 ± 0,3	0,1 ± 0,0	1,5 ± 0,9	nd	nd	nd	nd
	20-30cm	0,0 ± 0,0	0,4 ± 0,6	0,1 ± 0,1	1,3 ± 1,2	nd	nd	nd	nd

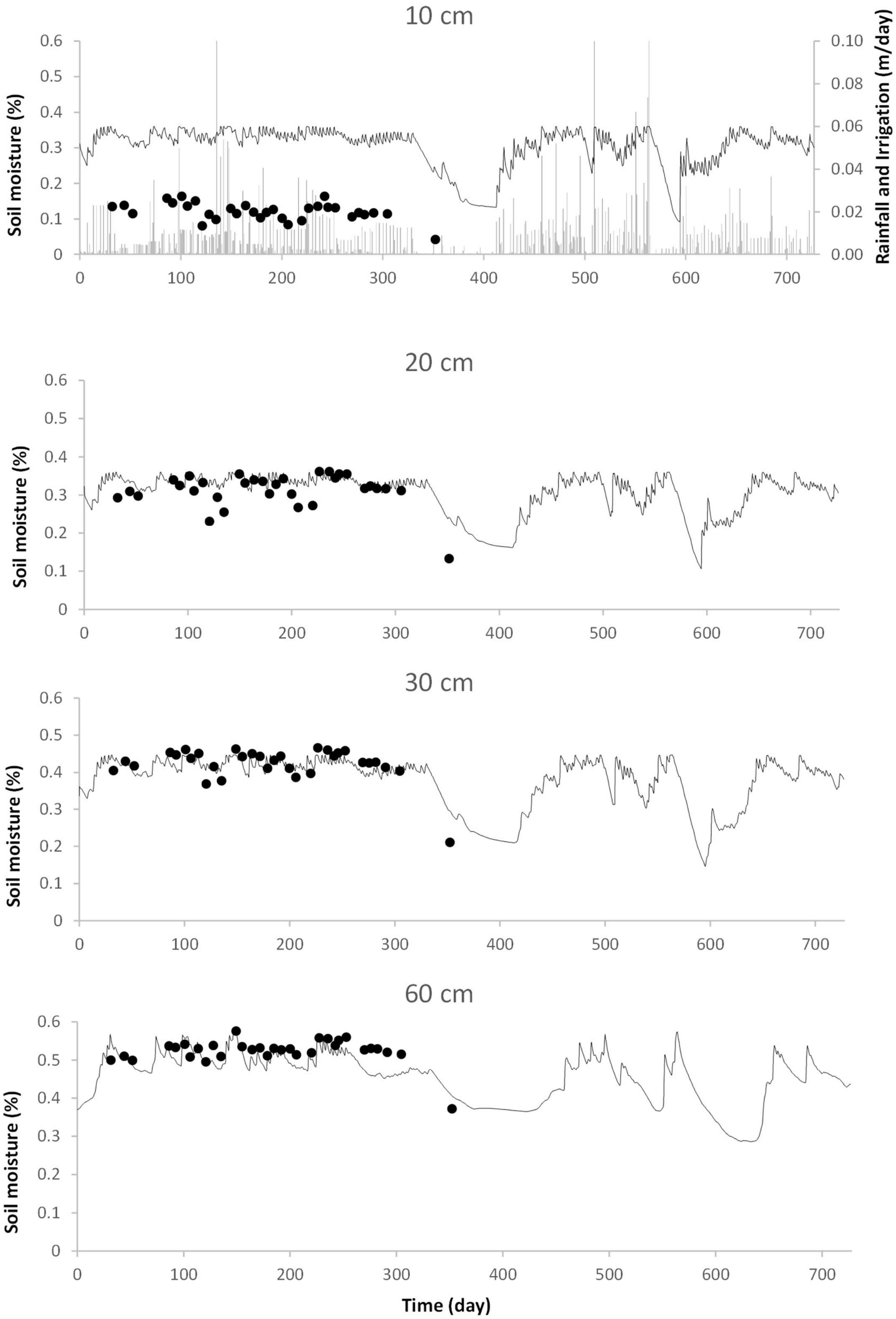

Figure S1. Soil moisture (%) measured in the SALSA trial (black point, first ratoon) or simulated with the Hydrus-1D model, at 10, 20, 30 and 60 cm depth. The bar indicated the daily rainfall and irrigation (mm d^{-1}).

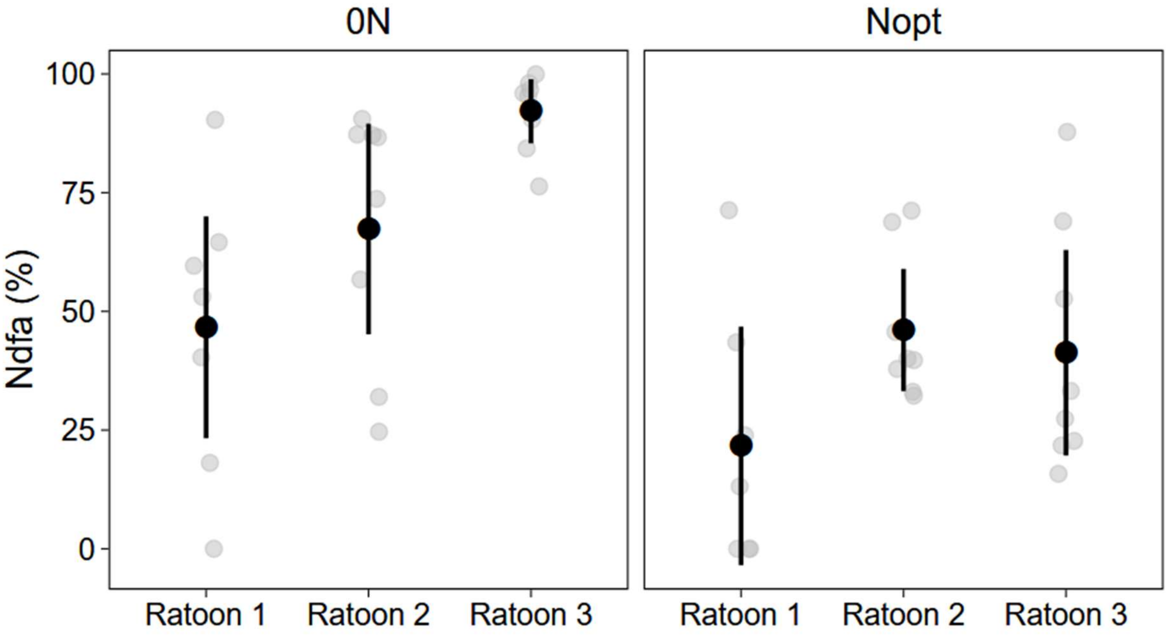

Figure S2. Nitrogen derived from the atmosphere (i.e. fixation) in the aboveground biomass of the legume at flowering in ratoon 1, 2, and 3, depending on the fertilization treatment (no urea fertilization, 0N, optimal urea fertilization Nopt).

Publications en co-auteur.

Ci-dessous sont fournis les premières pages des articles publiées, ainsi que la version préprint de l'article actuellement en révision.

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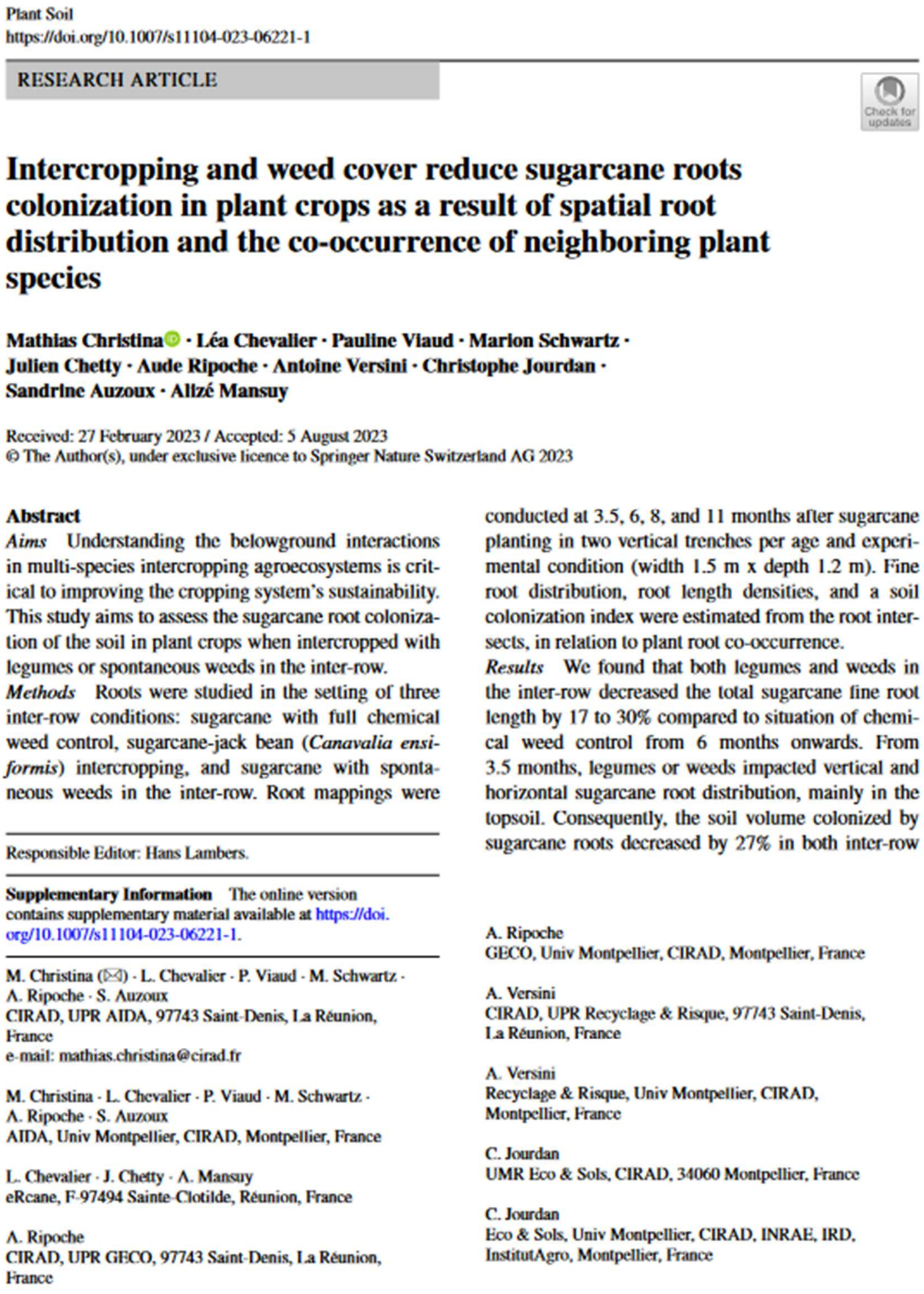

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RESEARCH ARTICLE

Intercropping and weed cover reduce sugarcane roots colonization in plant crops as a result of spatial root distribution and the co-occurrence of neighboring plant species

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Julien Chetty · Aude Ripoche · Antoine Versini · Christophe Jourdan ·
Sandrine Auzoux · Alizé Mansuy**

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Abstract
Aims Understanding the belowground interactions in multi-species intercropping agroecosystems is critical to improving the cropping system's sustainability. This study aims to assess the sugarcane root colonization of the soil in plant crops when intercropped with legumes or spontaneous weeds in the inter-row.
Methods Roots were studied in the setting of three inter-row conditions: sugarcane with full chemical weed control, sugarcane-jack bean (*Canavalia ensiformis*) intercropping, and sugarcane with spontaneous weeds in the inter-row. Root mappings were conducted at 3.5, 6, 8, and 11 months after sugarcane planting in two vertical trenches per age and experimental condition (width 1.5 m x depth 1.2 m). Fine root distribution, root length densities, and a soil colonization index were estimated from the root intersects, in relation to plant root co-occurrence.
Results We found that both legumes and weeds in the inter-row decreased the total sugarcane fine root length by 17 to 30% compared to situation of chemical weed control from 6 months onwards. From 3.5 months, legumes or weeds impacted vertical and horizontal sugarcane root distribution, mainly in the topsoil. Consequently, the soil volume colonized by sugarcane roots decreased by 27% in both inter-row

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Weed control under increasing cover crop diversity in tropical summer and winter

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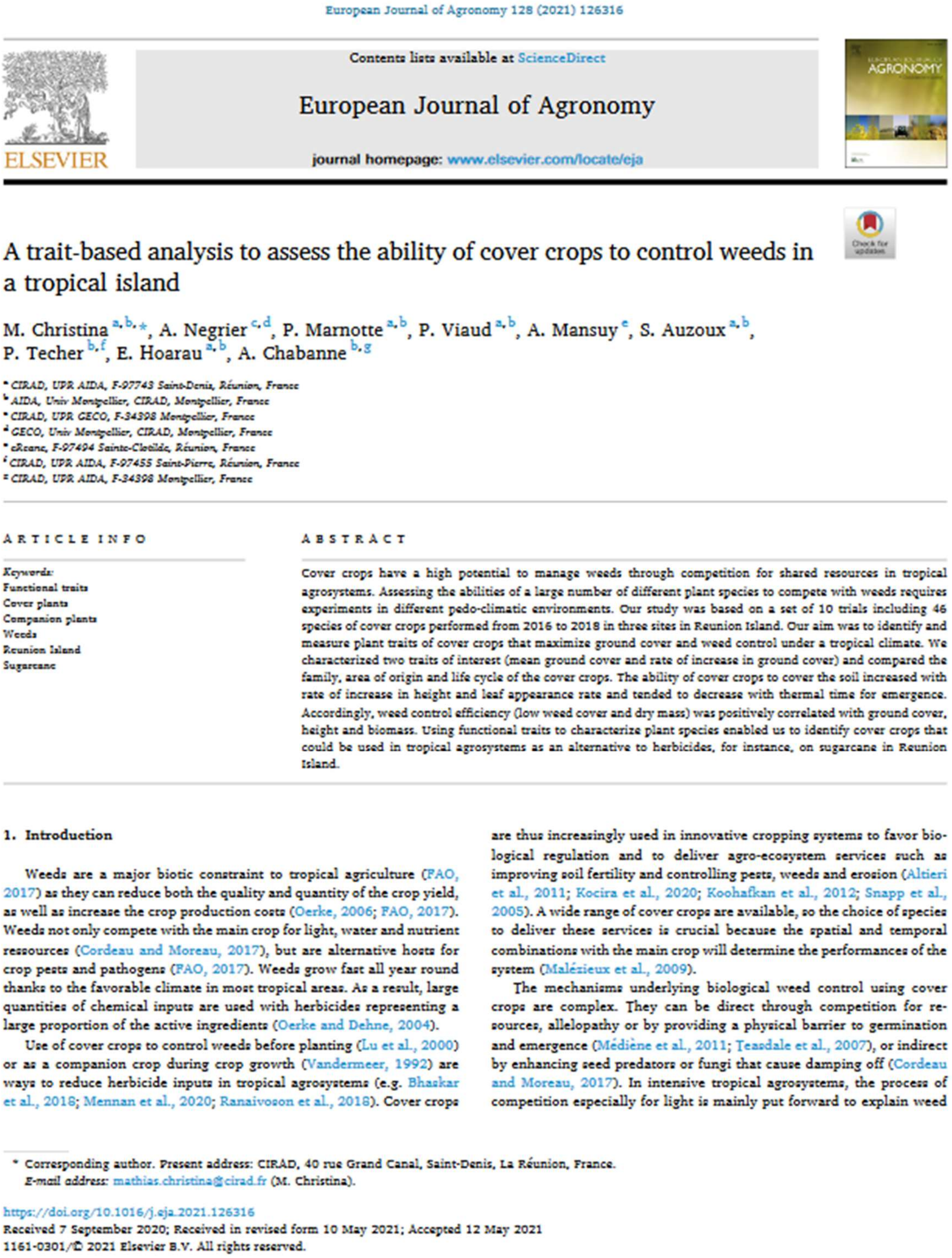
Description of the subject. Weed pressure is a main biotic constraint in tropical agriculture. Cover crop mixtures have increased in popularity to limit weed growth through competition for resources, but the relationship between cover crop diversity and weed suppression is still under debate.
Objectives. This study aimed to assess the impact of increasing cover crop diversity (one to four species) on weed control during two growing seasons (tropical summer and winter) in Reunion Island.
Method. Weed control was expressed regarding ground cover by weeds and weed aboveground dry mass in the mixtures during four months of growth and its response to cover crop traits was tested using structural equation models.
Results. While cover crops reduced weed ground cover and dry mass by 60% and 68% on average in summer and winter, respectively, a higher number of cover crop species within a mixture did not increase mean weed control. Nonetheless, weed control was influenced by the mixture composition and improved when including *Guizotia abyssinica*. Additionally, cover crop traits explaining weed control differed between growing seasons. In summer, weed control was mainly explained by the final cover crop aboveground biomass and leaf area (depletion strategy). In contrast, weed control was mainly explained by the cover crop rate of increase in ground cover (obstruction strategy) in winter.
Conclusions. Using traits to characterize cover crop mixture enables us to identify mixtures of species and traits adapted to different growing conditions. Our study suggests that particular attention on species identity rather than diversity should be paid in mixture to improve weed control in tropical conditions.
Keywords. Cover plants, mixed cropping, weed control, plant competition, tropical agriculture, Réunion.

Lutte contre les adventices dans le cadre d'une diversité croissante des cultures de couverture en été et en hiver dans les régions tropicales

Description du sujet. La pression des adventices est une contrainte biotique majeure dans les systèmes de culture tropicaux. Les mélanges de plantes de services ont gagné en popularité pour limiter la croissance des adventices par la compétition pour les ressources, mais la relation entre la diversité des plantes de services et la suppression des adventices est encore en débat.
Objectifs. Cette étude vise à évaluer l'impact de l'augmentation de la diversité des plantes de services (une à quatre espèces) sur la maîtrise des adventices pendant deux saisons de croissance (été tropical et hiver) sur l'île de la Réunion.
Méthode. La maîtrise des adventices a été exprimée en fonction de la couverture du sol et de la masse sèche aérienne des adventices, et la réponse de cette maîtrise aux traits des plantes de services a été testée en utilisant un modèle d'équations structurelles.
Résultats. Alors que les plantes de services ont réduit le recouvrement et la masse sèche des adventices de 60 % et 68 % en moyenne en été et en hiver, respectivement, un nombre plus élevé d'espèces dans un mélange n'a pas augmenté la maîtrise moyenne des adventices. Néanmoins, la maîtrise des adventices a été influencée par la composition du mélange et a été



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A trait-based analysis to assess the ability of cover crops to control weeds in a tropical island

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ABSTRACT

Cover crops have a high potential to manage weeds through competition for shared resources in tropical agrosystems. Assessing the abilities of a large number of different plant species to compete with weeds requires experiments in different pedo-climatic environments. Our study was based on a set of 10 trials including 46 species of cover crops performed from 2016 to 2018 in three sites in Reunion Island. Our aim was to identify and measure plant traits of cover crops that maximize ground cover and weed control under a tropical climate. We characterized two traits of interest (mean ground cover and rate of increase in ground cover) and compared the family, area of origin and life cycle of the cover crops. The ability of cover crops to cover the soil increased with rate of increase in height and leaf appearance rate and tended to decrease with thermal time for emergence. Accordingly, weed control efficiency (low weed cover and dry mass) was positively correlated with ground cover, height and biomass. Using functional traits to characterize plant species enabled us to identify cover crops that could be used in tropical agrosystems as an alternative to herbicides, for instance, on sugarcane in Reunion Island.

1. Introduction

Weeds are a major biotic constraint to tropical agriculture (FAO, 2017) as they can reduce both the quality and quantity of the crop yield, as well as increase the crop production costs (Oerke, 2006; FAO, 2017). Weeds not only compete with the main crop for light, water and nutrient ressources (Cordeau and Moreau, 2017), but are alternative hosts for crop pests and pathogens (FAO, 2017). Weeds grow fast all year round thanks to the favorable climate in most tropical areas. As a result, large quantities of chemical inputs are used with herbicides representing a large proportion of the active ingredients (Oerke and Dehne, 2004).

Use of cover crops to control weeds before planting (Lu et al., 2000) or as a companion crop during crop growth (Vandermeer, 1992) are ways to reduce herbicide inputs in tropical agrosystems (e.g. Bhaskar et al., 2018; Mennan et al., 2020; Ranaivoson et al., 2018). Cover crops are thus increasingly used in innovative cropping systems to favor biological regulation and to deliver agro-ecosystem services such as improving soil fertility and controlling pests, weeds and erosion (Altieri et al., 2011; Kocira et al., 2020; Koohafkan et al., 2012; Snapp et al., 2005). A wide range of cover crops are available, so the choice of species to deliver these services is crucial because the spatial and temporal combinations with the main crop will determine the performances of the system (Malézieux et al., 2009).

The mechanisms underlying biological weed control using cover crops are complex. They can be direct through competition for resources, allelopathy or by providing a physical barrier to germination and emergence (Médiène et al., 2011; Teasdale et al., 2007), or indirect by enhancing seed predators or fungi that cause damping off (Cordeau and Moreau, 2017). In intensive tropical agrosystems, the process of competition especially for light is mainly put forward to explain weed

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Title: Effect of crop management and climatic factors on weed control in sugarcane intercropping systems.

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Highlights

- Intercropping is a promising practice to reduce herbicide use in sugarcane.
- Weed control in tropical sugarcane intercropped systems was assessed over nine years.
- Intercropping was as efficient as chemical weeding to limit weed growth.
- Intercropping allowed to reduce herbicide use but increased the number of manual weedings.
- Weed control was influenced by cover crop and sugarcane management.

Abstract

Context

Cover crops in intercropping systems have a high potential to manage weeds in tropical agroecosystems as an herbicide alternative. However, it still needs to be better investigated in sugarcane cropping systems.

Objective

This study assesses the weed control ability of cover crops in sugarcane intercropping systems depending on crop management and climate in tropical conditions.

Methods

This study was based on a set of eight experiments, including ten cover crop species, performed from 2012 to 2021 in three areas in Reunion Island (France) and comparing three inter-row management treatments: i) low weed control, ii) chemical weed control and iii) cover crops sown in the inter-row. Mean weed and cover crop ground cover were measured during the entire crop cycle, and the complementary weed management practices (herbicide, manual weedings) were also recorded.

Results

Mean weed ground cover was reduced by 51 % in the cover crop and chemical treatments compared to low control. Additionally, the cover crop treatment had an herbicide treatment frequency index lower by 37% compared to chemical treatment but a higher number of manual weedings. In all treatments, weed cover increased with rainfall and temperature. In the cover crop treatment, a structural equation modeling approach allowed us to highlight the influence of cover crop (sowing date and species) and sugarcane management (crop cycle - plant or ratoon crop). Weed cover decreased with an increase in cover crop ground cover, which was higher for the early sowing date. Additionally, weed cover was lower in ratoon crops than plant crops but increased over time since intercropping was implemented.

Conclusions

This study highlights the predominance of management factors, compared with climate, to explain the effectiveness of weed control in sugarcane intercropping systems.

Implications

Due to the change in weed cover over time, there is a need to assess the long-term sustainability of this practice and the potential change in weed communities.

Keywords: *Saccharum officinarum*; companion plants; interplanting; living mulch; weed management

1. Introduction

Over the past few decades, the use of pesticides in agriculture has surged worldwide, mainly due to the expansion of agricultural land and increased application rates (Brühl and Zaller, 2022). However, concerns about pesticides' environmental and health impacts have spurred efforts to explore alternative weed management strategies (FAO, 2019). The emergence of herbicide-resistant weeds is particularly concerning, which poses a significant challenge to weed control (Heap, 2014). In this context, research and development efforts have focused on evaluating non-chemical approaches to weed management (Jacquet et al., 2022).

In tropical and temperate climates, weeds are a significant biotic constraint on agricultural productivity, reducing crop yield and increasing production costs (FAO, 2017; Oerke, 2006). In recent years, the use of cover crops in intercropping systems gained attraction as a promising alternative to herbicides for weed control while also delivering other benefits, such as improved soil fertility and erosion control (Garcia et al., 2018; Gu et al., 2021; Kocira et al., 2020; Scavo et al., 2022; Verret et al., 2017). There is broad agreement that a vigorous cover crop will suppress weeds and reduce herbicide use (Bhaskar et al., 2018; Christina et al., 2021b; Mennan et al., 2020; Ranaivoson et al., 2019).

Sugarcane is a globally significant crop, serving as the primary source of sugar, ethanol, and electric energy in tropical and sub-tropical regions (de Miranda and Fonseca, 2020). Given the increasing demand for ethanol and electricity as potential substitutes for fossil fuels to reduce greenhouse gas emissions (Goldemberg et al., 2014), the sustainability of sugarcane production came under scrutiny (Bordonal et al., 2018), particularly concerning herbicide use. While several studies have highlighted the potential benefits of intercropping sugarcane with cover crops to diversify production (Geetha et al., 2019), increase economic returns per unit of land (Rehman et al., 2014), enhance soil fertility (Solanki et al., 2017), or reduce fertilizer inputs (Prellwitz and Coelho, 2011; Tian et al., 2020), only a limited number have examined the effectiveness of cover crops for inter-row weed management (e.g., Geetha et al., 2019). Although many sugarcane cultivars are now resistant to pests and pathogens (Sundar et al., 2015), weed management remains a major production constraint (Suganthi et al., 2019).

As shown in temperate systems, various climatic and management constraints influence intercropping performance for weed control (Gardarin et al., 2022). In the case of tropical sugarcane systems, climatic factors such as temperature and precipitation could affect the growth and development of both the sugarcane, the cover crop, and the weeds, potentially altering the timing and intensity of weed competition. Also, management factors such as mulching (Carvalho et al., 2017) and cover crop management practices could affect weed control in sugarcane intercropping systems. For example, the choice of cover crop species and the timing of its planting can affect its competitiveness with weeds and its ability to suppress weed growth (Dayoub et al., 2022; Liebman and Dyck, 1993). Additionally, weed control in intercropping systems often relies on complementary pre-emergent herbicide application before cover crop sowing and manual weed control (Mansuy et al., 2019), which must be considered to assess the ability of cover crops to manage weeds in the inter-row. Thus, to optimize the performance of sugarcane intercropping systems for weed control, it is essential to consider and address the range of climatic and technical constraints that may influence their effectiveness.

Sugarcane is the dominant agricultural industry on Reunion Island, with more than half of agricultural land devoted to it, making it a crucial economic, social, and environmental resource

(Leung, 2015). The island's tropical climate is favorable to weed growth, with a daily delay in weed control resulting in yield losses of 0.1-0.2 Mg ha^{-1} and an absence of weed control leading to 80% yield loss (Marnotte et al., 2008). As a result, chemical weed control is the primary strategy used to control weeds, making the sugarcane sector the largest consumer of herbicides on the island (Antoir et al., 2016). However, the withdrawal of many chemical herbicides and government incentives to reduce the use of pesticides by 50% by 2025 under the Ecophyto program have prompted the sector to explore alternative weed control methods (Antoir et al., 2016). While the efficacy of cover crops for weed control in sugarcane inter-rows has been recently tested on Reunion Island (Mansuy et al., 2019), the impact of the island's high spatial climate variability remains poorly understood.

A better understanding of the factors influencing weeds x cover crops x sugarcane interactions in intercropping is crucial for developing effective strategies to reduce herbicide use while maintaining yield (Viaud et al., 2023), particularly in tropical islands with diverse environmental conditions. The objectives of this study were to i) assess the weed response to sugarcane intercropping compared to monocropping, ii) assess the changes in herbicide application and manual weeding practices in the intercropping system compared to monocropping, and iii) disentangle the effects of the cover crop, the complementary weed control management, the climate, and the cover crop management on weeds in the sugarcane inter-row. We gathered data from a network of 8 intercrop experiments performed on Reunion Island from 2012 to 2021 to achieve these objectives.

2. Material & Methods

2.1. Experimental network

Reunion Island is located in the Southwest of the Indian Ocean near Madagascar. Sugarcane agricultural areas are located from sea level to 800 m a.s.l, in dry (West Coast) and high rainfall conditions (East Coast, Christina et al., 2021a). Eight field experiments were conducted over nine years (2012-2021) in three locations on Reunion Island (Table 1). Five experiments were conducted on-station in the North of the Island on a Nitisol (WRB classification). Three experiments were conducted on-farm, one in the East on a Ferralsol and two in the West on an Andic Cambisol. The length of each experiment varied between one to seven years. In this study, a trial defines an experiment performed in a specific year (28 trials). Mean annual temperatures (T_{MEAN}) varied from 19.6 to 26.1°C, and annual rainfall varied from 718 to 2611 mm y^{-1}, depending on trials. Soil chemical characteristics were measured at each experiment's beginning and listed in Table A1. The weather variables were obtained from the Meteor software (https://smartis.re/METEOR), which interpolates daily weather variables from the Meteo France and CIRAD weather station network.

2.2. Experimental design and sugarcane management

Each experiment consisted of a randomized block sugarcane (*Saccharum officinarum*) to compare three inter-row management treatments: a weedy treatment with low weed control in the sugarcane inter-row (LC), a treatment with a cover crop sown each year in the inter-row (CC) and a treatment with chemical weed control in the whole plot (CH). Depending on the experiments, each treatment was repeated in two to four blocks, with each elementary plot varying from 60 to 780 m^2 (Table A2). The sugarcane was planted with a 1.5m row distance in all experiments. All experiments were planted with the sugarcane R579 cultivar except for the “P12” experiment using the R587 cultivar (Table A2). All experiments were irrigated except for “Canecoh_OF”, located in the East rainy part of the island. Mineral NPK fertilization was supplied to reach the optimum yield according to recommendations provided by the Serdaf software based on soil analysis (Versini et al., 2018). NPK fertilization varied depending on trials, with an average of 180 kg N ha^{-1}, 100 kg P ha^{-1}, and 250 kg K ha^{-1}. In most trials, fertilization has been split with a first application one month after planting or harvest and a second at 2-3 months (details available in the data paper Ngaba et al., 2023).

Table 1. Description of the experimental network used in this study with the experiment location, trial harvest year (including crop cycle, P = plant crop, R1 = first ratoon crop, R2 = second ratoon crop…), cover crop species sown in the trial and their sowing dates (months after planting or harvest, MAP/H), mean temperature (TMEAN, °C) and rainfall (mm y^{-1}) over the sugarcane cycle, and trial identification (ID). Different sowing dates in the same trial indicated different sowing date treatments. In the cover crops species, “Mixture” indicated a mixture of cover crops (detailed in Table A3).

Location, Experiment (Latitude, Longitude, Altitude)	Harvest year	Cover crop species	Sowing date (MAP/H)	TMEAN (°C)	Rainfall (mm y^{-1})	ID
North	2018 (P)	*Vigna unguiculata*	2.8	26.0	1421	1
“P12”	2019 (R1)	*V. unguiculata*	1.6	25.0	1260	2
(-20.903, 55.532, 70m a.s.l)	2020 (R2)	*V. unguiculata*	1.6	23.9	1284	3
North	2015 (P)	*Desmodium intortum, Canavalia ensiformis*	1.1/ 2.3/ 3.3	24.0	2008	4
“P41”	2016 (R1)	*D. intortum, C. ensiformis*	2.3	24.2	1117	5
(-20.903, 55.528, 68m a.s.l)	2017 (R2)	*Guizotia abyssinica, C. ensiformis, Avena sativa, V. unguiculata, Crotalaria juncea, Crotalaria spectabilis*	2.3	24.2	1350	6
	2018 (R3)	*G. abyssinica, C. ensiformis, Brassica carinata, V. unguiculata, C. juncea, C. spectabilis*	1.6	25.0	1528	7
	2019 (R4)	*G. abyssinica, C. ensiformis, B. carinata, V. unguiculata, C. juncea, C. spectabilis*	1.1	25.6	1327	8
	2020 (R5)	*G. abyssinica, C. ensiformis, B. carinata, V. unguiculata, Raphanus sativus*, Mixture	2.6	25.0	718	9
	2021 (R6)	*G. abyssinica, C. ensiformis, B. carinata, V. unguiculata, R. sativus*, Mixture	1.38	24.1	1070	10
North	2018 (P)	*C. ensiformis*, Mixture	2,7	24.4	1527	11
“P25_montagne”	2019 (R1)	*C. ensiformis*, Mixture	1.3	25.6	1310	12
(-20.902, 55.531, 50m a.s.l)	2020 (R2)	*C. ensiformis*, Mixture	1.3	25.0	736	13
North	2012 (P)	*V. unguiculata, C. ensiformis, Dolichos lablab, D. intortum, Cajanus cajan*	0.4 /1.9	23.9	1569	14
“P25_LEG”	2013 (R1)	*V. unguiculata, C. ensiformis, D. intortum*	0.7 /1.8	24.4	1418	15
(-20.902, 55.531, 50m a.s.l)	2014 (R2)	*V. unguiculata, C. ensiformis, D. intortum*	0.5 /2	24.4	1023	16

	2015 (R3)	*V. unguiculata, C. ensiformis, D. intortum*	0.7 /1.6	24.3	1941	17
	2016 (R4)	*V. unguiculata, C. ensiformis*	1.8	24.1	1063	18
North “P25_ITK” (-20.902, 55.531, 50m a.s.l)	2013 (R1)	*V. unguiculata*	0.8	24.2	1103	19
	2014 (R2)	*V. unguiculata*, Mixture	0.6 /2.3	24.5	1033	20
	2015 (R3)	*B. juncea*, Mixture	0.7 /1.6	24.4	1974	21
East “Canecoh_OF” (-21.047, 55.682, 170m a.s.l)	2016 (R4)	*V. unguiculata, C. ensiformis*	1.5	22.8	1218	22
	2017 (P)	*C. ensiformis*, Mixture	3	22.8	1505	23
	2018 (R1)	*C. ensiformis*, Mixture	1.6	22.6	1289	24
	2019 (R2)	*C. ensiformis*, Mixture	2.1	22.9	2501	25
	2020 (R3)	*C. ensiformis*, Mixture	1.5	22.4	2611	26
West “Ecocanne_AC” (-21.067, 55.280, 545m a.s.l)	2015 (R1)	*D. intortum, C. ensiformis*	0 /1.3	20.3	1241	27
West “Ecocanne_OB” (-21,066, 55,285, 575m a.s.l)	2014 (R2)	*D. intortum, C. ensiformis*	0.3 /1.4	19.6	860	28

2.3. Weed management, HTFI, and manual weedings

Weed management of all treatments included: the application of a pre-emergent herbicide after sugarcane planting or harvest in the whole plot, a manual or chemical weeding of the sugarcane row to limit the impact on sugarcane production (throughout the crop cycle), and a specific manual weeding of the weeds considered as the most harmful in the whole plot (in particular, tall grasses: *Panicum Maximum* and *Rottboellia cochinchinensis*). In the chemical control treatment, additional post-emergent herbicides were systematically applied to limit ground cover by weeds below the threshold of 30% ground cover (sugarcane sector recommendations). Additional manual weedings were occasionally performed in the cover crop treatment when ground cover by weeds exceeded 30%. An Herbicide Treatment Frequency Index (HTFI) was calculated as the sum of the ratio of the herbicide dose applied divided by the recommended dose across each herbicide application in a specific plot (Halberg, 1999). The HTFI was only available in 81% of trials. Details of the chemical compounds used for herbicides are available in the data paper (Ngaba et al., 2023). Information regarding manual weedings included: manual weeding in the row only, manual weeding in the inter-row only, and manual weeding in the whole plot. In the present study, the number of manual weedings in the inter-row (MW_{IR}) was considered as the sum of those in the whole plot and the inter-row, while the number of manual weedings in the row (MW_R) was considered as the sum of those in the whole plot and sugarcane row. The number of manual weedings was only available in 89% of trials. In most cases, when an herbicide was applied, or a manual weeding was performed during the trials, all plots from the same inter-row management treatment were concerned. Consequently, the mean values per treatment and trial were used in the following analyses.

2.4. Cover crop management

The experimental network tested ten cover crop species (Table 2). They included six Fabaceae, three Brassicaceae, and one Asteraceae plant, as well as mixtures (Table A3). Cover crops were sown manually or mechanically in two to three lines depending on trials (Table A3). The sowing date depended on trials (Table 1, Table A3). At the end of their development, cover crops died gradually with sugarcane closure or were mechanically destroyed before canopy closure in a few treatments (14% of treatments, Table A3). The sowing densities were determined based on previous studies and literature (Christina et al., 2021b; Table A3). In mixtures, the sowing density of each cover crop was its density in pure crop divided by the number of present species (Négrier et al., 2023).

Table 2. Cover crop species sown in the experimental network: species, common name, family, area of origin, and the number of trials including these species (Ntrial) is indicated.

Species	Common name	Family	Origin	Ntrial
Brassica carinata	Abyssinian mustard	Brassicaceae	Temperate	4
Brassica juncea	Brown mustard	Brassicaceae	Temperate	1
Canavalia ensiformis	Jack bean	Fabaceae	Tropical	19
Crotalaria juncea	Sunn hemp	Fabaceae	Tropical	2
Crotalaria spectabilis	Showy crotalaria	Fabaceae	Tropical	2
Desmodium intortum	Greenleaf	Fabaceae	Tropical	6
Dolichos lablab	Hyacinth bean	Fabaceae	Tropical	1
Guizotia abyssinica	Niger	Asteraceae	Temperate	4
Raphanus sativus	Radish	Brassicaceae	Temperate	2
Vigna unguiculata	Cowpea	Fabaceae	Tropical	13
Mixture of cover crops	Detailed in Table A3			10

2.5. Ground cover by cover crop and weeds

The ground cover by cover crops and weeds was estimated in each elementary plot (excluding borders) using a visual notation method according to a rating scale ranging from 0 to 100% and described in Table A4. This method makes it possible to assess the ground cover by species in multi-species crops and was used in previous studies (Christina et al., 2021b; Mansuy et al., 2019; Marnotte, 1984; Négrier et al., 2023). In the trial plots, ground cover by i) cover crops and ii) weeds was performed every month during the first six months and then every two months up to the sugarcane harvest. Ground cover was then linearly interpolated between two dates of measurement to calculate a mean ground cover by cover crops (COV_{CC}) or weeds (COV_{WEED}) between two sugarcane harvest dates. The current study will not present the ground cover results by weed species. Nonetheless, a description of weed species present in the trials is available in Table A5.

2.6. Data analysis

Prior to analysis, a few elementary plots were removed due to cover crop failure due to not tested factors: accidental herbicide application in the inter-row (14 out of 607 plots), no emergence due to irrigation failure or excessive water stress (60 out of 607 plots), cover crop destruction due to bio-aggressors (6 out of 607 plots). All analyses were performed using R 4.1 (R Development Core Team, 2022).

For all statistical analyses, the following procedure was made. If necessary, to ensure residue normality, the target variable was transformed using the Box-Cox function (powerTransform function from car R package, Fox et al., 2023) based on a Gaussian law. Then, the identification of the random effects (among trial identification, location, year, and nested effects) was realized by comparing the linear mixed model (lme function, nlme R package, Pinheiro et al., 2022) and the only fixed model (gls function, nlme R package) fitted using the restricted maximum likelihood (REML) estimation and compared using the Bayesian information criterion (BIC). Then, the fixed effect selection was performed using a stepwise downward procedure based on BIC from the model with all fixed effects and interactions (lme function) and fitted using the maximum likelihood (ML) estimation (Zuur et al., 2009). Finally, the model parameters of the resulting model were fitted using REML estimation. The assumptions of homogeneity and normality were checked graphically using histograms of residuals, quantile-quantile plots of Pearson's residuals versus standardized Gaussian sample, and plots of residuals against fitted values. Pairwise comparisons among treatments were performed using the emmeans function with a Tukey p adjustment method and a 0.05 probability threshold (emmeans R package, Lenth et al., 2023). The models' mean predicted and confidence interval values depending on factors were calculated using the emmeans function.

In the first step, the influence of the inter-row treatment (low control, cover crop, or chemical), the experiment identification, the mean temperature (T_{MEAN}), the cumulated rainfall over the crop cycle, the crop cycle (plant or ratoon crop), the irrigation management, and their interaction on COV_{WEED} (transformation λ=0.39) were tested as fixed effects using a linear mixed model with the trial identification (ID) as a random effect. The same analysis was performed for HTFI (transformation λ=0.46) and the number of manual weedings in the inter-row (MW_{IR}, transformation λ=0.46) and in the row (MW_R, transformation λ=0.50). Note that analyses on MW_{IR} and MW_R were also realized without transformations with a generalized linear mixed model (glmer function) with a Poisson law which gave the same results. The linear analyses with a Gaussian law and transformation were chosen for these two variables to be applied in the following structural equation model.

In the cover crop treatment, COV_{WEED} could result from the influence of different weed control management: COV_{CC}, MW_{IR}, and HTFI, as well as other biotic and abiotic factors. COV_{CC}, MW_{IR}, and HTFI could themselves be influenced by climate and management through weed pressure. Consequently, the influence of climate (T_{MEAN} and Rainfall), sugarcane management (crop cycle, irrigation, number of years since the implementation of intercropping - Y_{IC}), cover crop (CC) management (CC species - $CC_{species}$ and sowing date – CC_{Sowing_date}), MW_{IR}, and HTFI on COV_{CC} and COV_{WEED} was tested using a mixed structural equation model (SEM, psem function from the piecewiseSEM R package, Lefcheck, 2016) to take into account the indirect effect of factors on COV_{WEED} through COV_{CC}, HTFI, and MW_{IR}. The initial SEM included four submodels (hypotheses):

$$COV_{WEED} \sim COV_{CC} + MW_{IR} + HTFI + Y_{IC} + T_{MEAN} + Rainfall + CC_{species} + CC_{Sowing\_date} + Cycle + Irrigation, \quad random = 1|ID$$

$$COV_{CC} \sim MW_{IR} + Y_{IC} + T_{MEAN} + Rainfall + CC_{species} + CC_{Sowing\_date} + Cycle + Irrigation, \quad random = 1|ID$$

$$HTFI \sim Y_{IC} + T_{MEAN} + Rainfall + CC_{species} + CC_{Sowing\_date} + Cycle + Irrigation, \quad random = 1|ID$$

$$MW_{IR} \sim Y_{IC} + T_{MEAN} + Rainfall + CC_{species} + CC_{Sowing\_date} + Cycle + Irrigation, \quad random = 1|ID$$

Following a similar procedure to the linear mixed model (Box-Cox transformation, ML estimation for fixed effect selection, and REML estimation for the final parameter estimations), insignificant effects (α > 0.05), as well as unlinked variables, were progressively removed up to reach the global goodness-fit-criteria (Fisher's C test).

2.7. Data availability

All the data, including observations and sugarcane management, were described in a companion paper (Ngaba et al., 2023), and are freely available in the CIRAD dataverse "Agroecological Practices to reduce wEED infestAtion in the tropIcS" (APEEDAIS, https://dataverse.cirad.fr/dataverse/APEEDAIS). The dataset links separated per trial were listed in supplementary materials.

3. Results

3.1. COV_{WEED} response to inter-row treatment, climate, and sugarcane management

Mean annual ground cover by weeds (COV_{WEED}) varied from 0 to 75% depending on trial and inter-row treatment (Fig. 1). COV_{WEED} was influenced by the inter-row treatment ($p<0.0001$), T_{MEAN} ($p=0.0091$), the total rainfall ($p=0.0127$), the crop cycle ($p=0.0021$), the interactions between the treatment and the rainfall ($p<0.0001$), and the interaction between the experiment and the inter-row treatment ($p=0.0068$, Table A6). On average, COV_{WEED} was reduced by 51% in both cover crop and chemical treatments, compared to low control treatment (Table A7, Fig. 1a). Nonetheless, COV_{WEED} was not influenced by the inter-row treatment in two experiments (P25_ITK and Ecocanne_OB) out of eight, and all treatments were similar in both experiments (Table A8). COV_{WEED} was 43% lower in ratoon crops than in plant crops, with mean values of 13% and 23% in ratoon crops (characterized by a sugarcane trash mulch cover) and plant crops (a first-year without mulch cover), respectively (Table A6, Fig 1b). COV_{WEED} increased with the total rainfall over the crop cycle in both low control and chemical treatment but not in the cover crop treatment (Table A6, Fig. 1c). COV_{WEED} increased with T_{MEAN} in all treatments (Table A6, Fig. 1d).

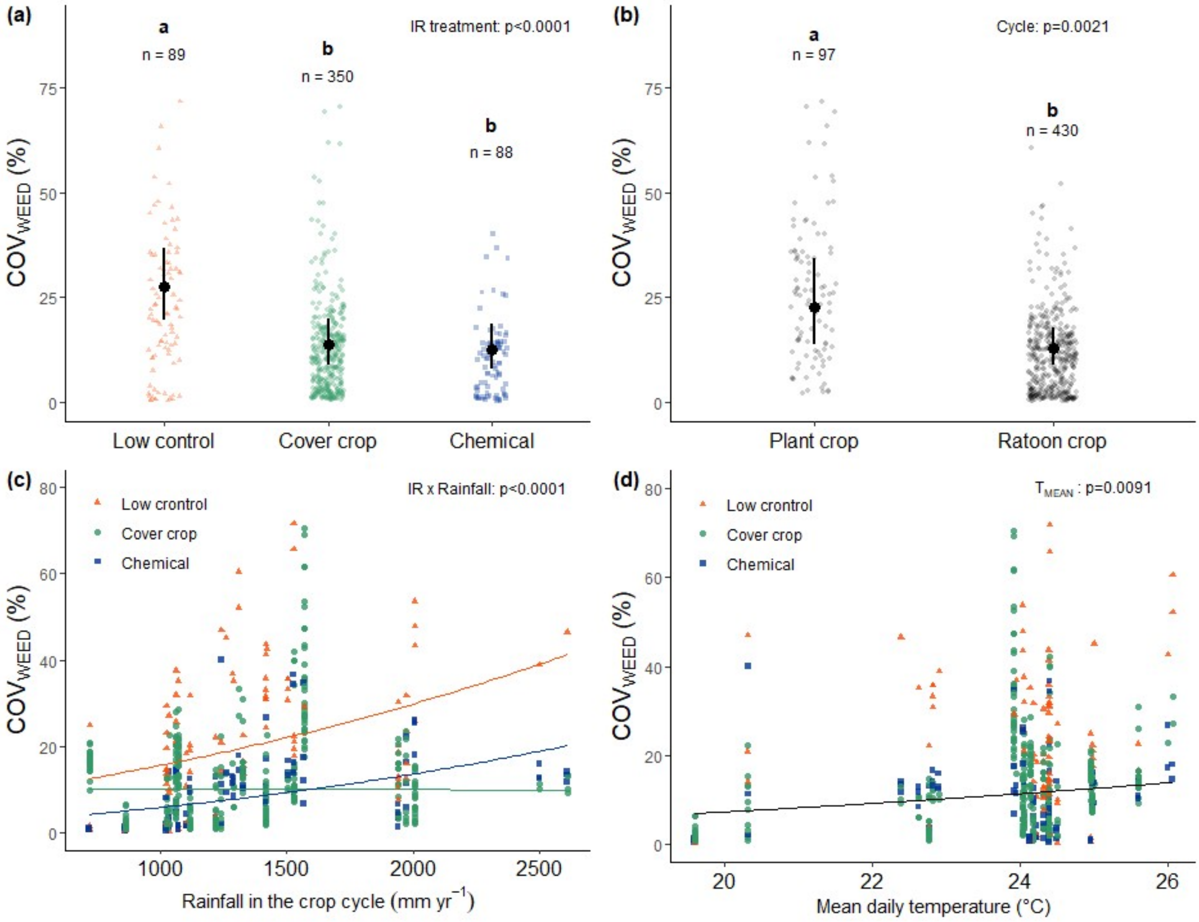

Fig. 1. Mean ground cover by weeds (COV_{WEED}) response to inter-row management treatment (IR, a), sugarcane crop cycle (plant or ratoon crop, b), rainfall (c), and mean daily temperature (T_{MEAN}, d) during the crop cycle. In (a) and (b), the mean values (black point) and confidence intervals (black line) predicted by the mixed model with the trial as a random effect are presented. The number of measurements in each category is indicated by "n". Letters indicated significant differences in the pairwise comparisons (Table A7). In (c) and (d), the lines represented the predicted response by the model after back-transformation. P values of the fixed effects in the variance analysis are indicated (Table A6).

3.2. HTFI and manual weedings response to inter-row treatment

The herbicide frequency treatment index (HTFI) and the number of manual weedings in the sugarcane inter-row (MW_{IR}) and in the row (MW_R) were influenced by the inter-row treatment (Fig. 2, Table A6). The HTFI was reduced by 37% in the cover crop and low control treatments compared to chemical treatment (Fig. 2a). Nonetheless, the number of manual weedings in the cover crop treatment increased by 86% and 34% in the inter-row and row, respectively (Fig. 2b,c), compared to both low control and chemical treatments. No climate or sugarcane management influences were observed on HTFI, MW_{IR}, and MW_R.

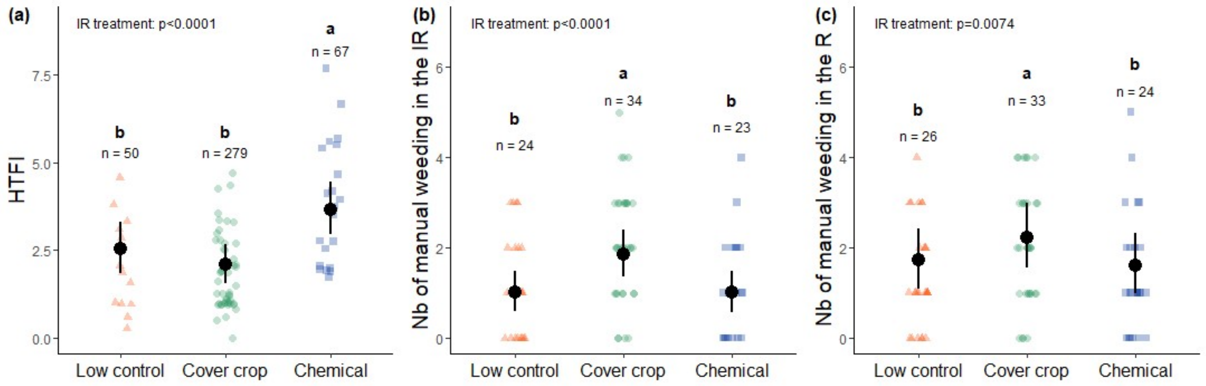

Fig. 2. Herbicide treatment frequency index (HTFI, a) and number (nb) of manual weedings in the inter-row (IR, b) and row (R, c) depending on the inter-row management treatment. The mean values (black point) and confidence intervals (black line) predicted by the linear mixed models with the trial as a random effect are presented. The number of measurements in each category is indicated by "n". Letters indicated significant differences in the pairwise comparisons (Table A7). P values of the fixed effects in the variance analysis are indicated (Table A6).

3.3. Structural equation models on COV_{CC} and COV_{WEED}

In the cover crop treatment, COV_{WEED} was directly influenced by COV_{CC}, the crop cycle, the number of years since the implementation of intercropping (Y_{IC}), the cover crop sowing date and species, and COV_{CC} was directly influenced by cover crop sowing date, species, and crop cycle (Fig. 3b, Table A9, A12). COV_{WEED} was also indirectly influenced by cover crop sowing date and species through COV_{CC}. These retained paths respected the Fisher global goodness-of-fit criteria in the SEM with all the data (Fischer C = 0.76, p=0.94, Table A9) as well as in SEM using only plant crop data (Fischer C = 2.9, p=0.23, Table A10) and ratoon crop data (Fischer C = 3.9 p=0.42, Table A11). These fixed factors explained 49% and 34% of COV_{WEED} and COV_{CC} variability, respectively (Fig. 3b). Nonetheless, the respective influence of each factor depended on the crop cycle (Fig. 3c,d). In plant crops, the cover crop sowing date affected COV_{CC} while not in ratoon crops, and this factor had a prominent effect on COV_{WEED} in plant crops compared to ratoon crops (see estimates in Table A10 and A11). The cover crop species directly influenced COV_{WEED} in ratoon crops but not plant crops.

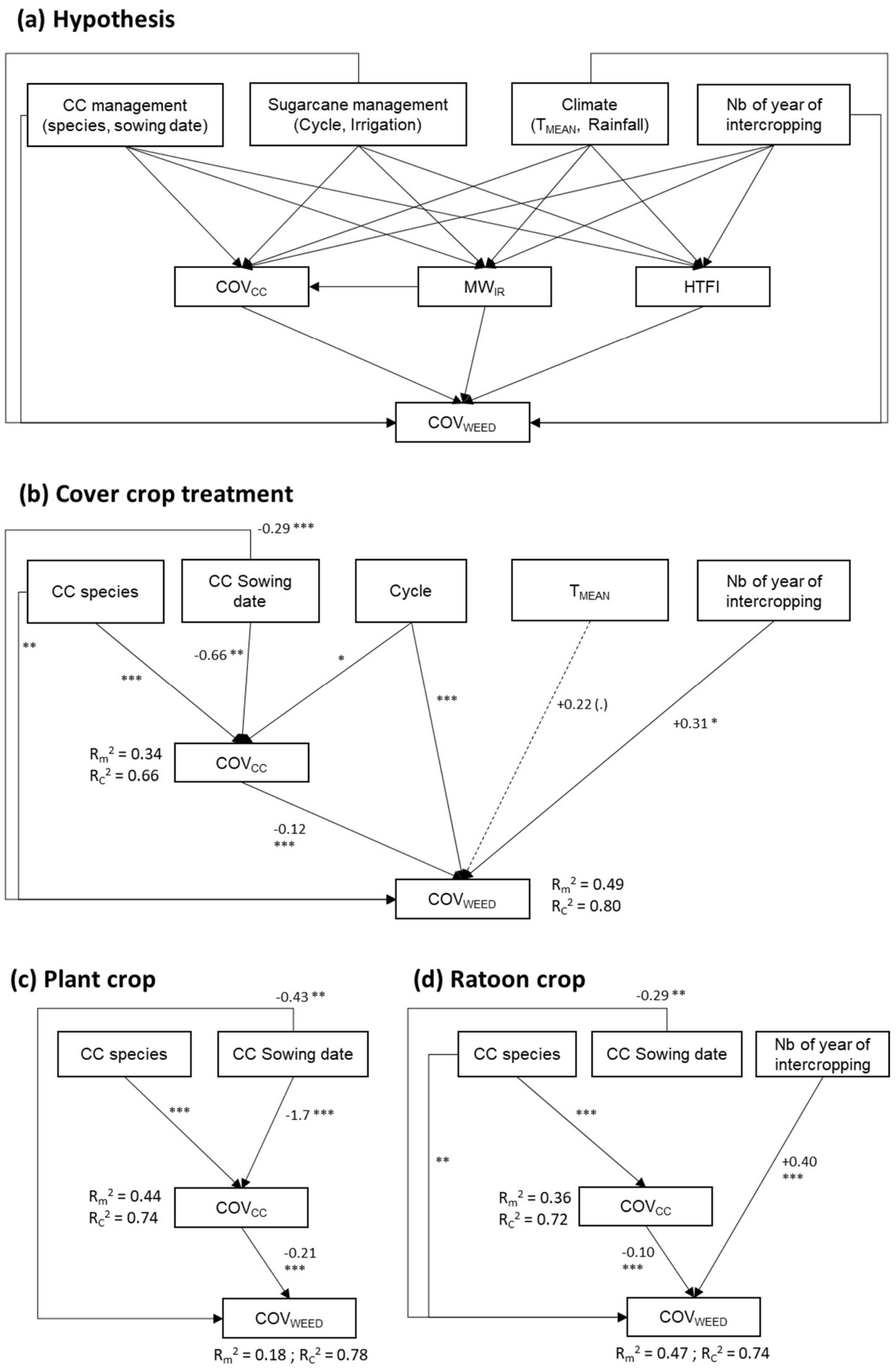

Fig. 3. Structural equation model (SEM, a) showing direct and indirect effects of cover crop management, weed management, and environmental conditions on the ground cover by weeds (COV_{WEED}) and cover crop (COV_{CC}) in the cover crop treatment considering the hypothesis (a), final SEM with all data (b), plant crop data (c) or ratoon crop data (d). Arrows represent the structural equation with variables: mean annual temperature (T_{MEAN}), rainfall, crop cycle, irrigation management, cover crop (CC) species, and sowing date, number (nb) of years since the implementation of intercropping, the number of manual weedings in the inter-row (MW_{IR}), and the herbicide treatment frequency index (HTFI). In (b), (c), and (d), the arrows represent the significative result of the analysis. The mode estimates are given for quantitative variables (after Box-Cox transformation), and p values are indicated by ***, **, *, and (.) when lower than 0.001, 0.01, 0.05, and 0.1, respectively. Marginal R^2 (R_m^2) and conditional R² (R_C^2) are given. Details on model estimates for qualitative variables and variance analyses are given in Supplementary Material (Table A9 to A11).

3.4. Weeds response to cover crops growth and species

A negative impact of COV_{CC} on COV_{WEED} was shown in the structural equation model in both plant and ratoon crops (Fig. 3). The SEM predicted a decrease in COV_{WEED} from 39 to 14% in plant crops, and 14 to 5% in ratoon crops, with increasing COV_{CC} (Fig. 4a). COV_{CC} was influenced by the cover crop species in both plant and ratoon crops (Fig. 3, 5a). In contrast, COV_{WEED} was only influenced by the cover crop species in the ratoon crops (Fig. 3, 5b). In plant crops, the highest COV_{CC} were found in cover crop mixture (56%), and *V. unguiculata* (49%), followed by *C. ensiformis* (42%), and the lowest COV_{CC} was found in *D. intortum* (29%, Fig. 5a). In ratoon crops, cover crop species with the highest COV_{CC} was *C. ensiformis* (35%) followed by *C. juncea* (33%), *C. spectabilis* (29%) and *R. sativus* (28%). The lowest COV_{CC} were found for *G. abyssinica* (11%), *V. unguiculata* (13%), and *B. juncea* (near 0%). In ratoon crops, COV_{WEED} was similar among all CC species except for *C. ensiformis*, which has a lower COV_{WEED} (8%) than *V. unguiculata* (11%, Fig. 5b).

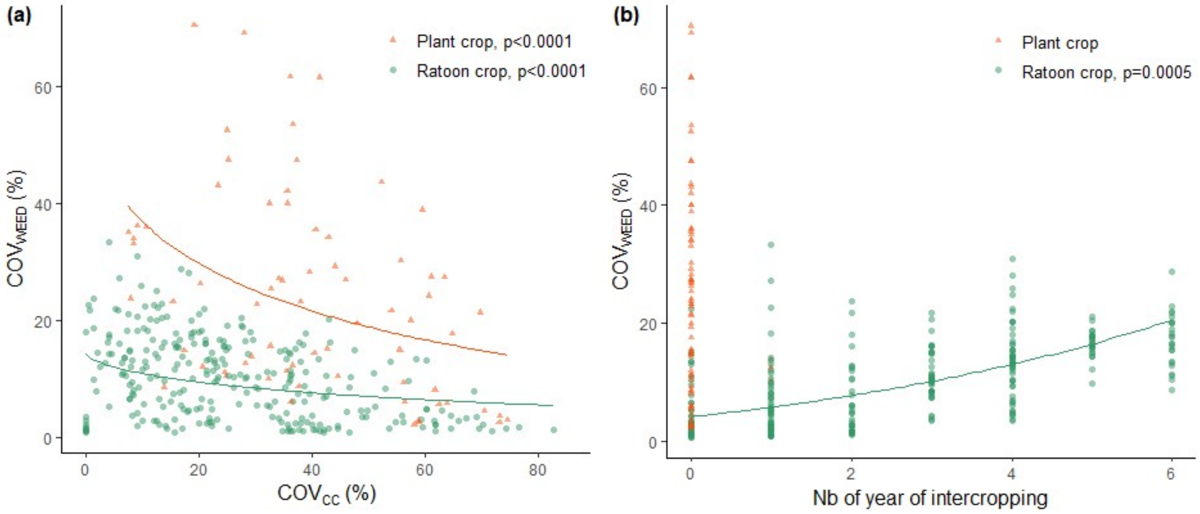

Fig. 4. Mean ground cover by weeds (COV_{WEED}) in the cover crop treatment in the inter-row in response to the mean ground cover by cover crop (COV_{CC}, a) and the number (Nb) of years since the implementation of intercropping (b). The lines represented the predicted response by the SEM models after back-transformation, and the P values are indicated.

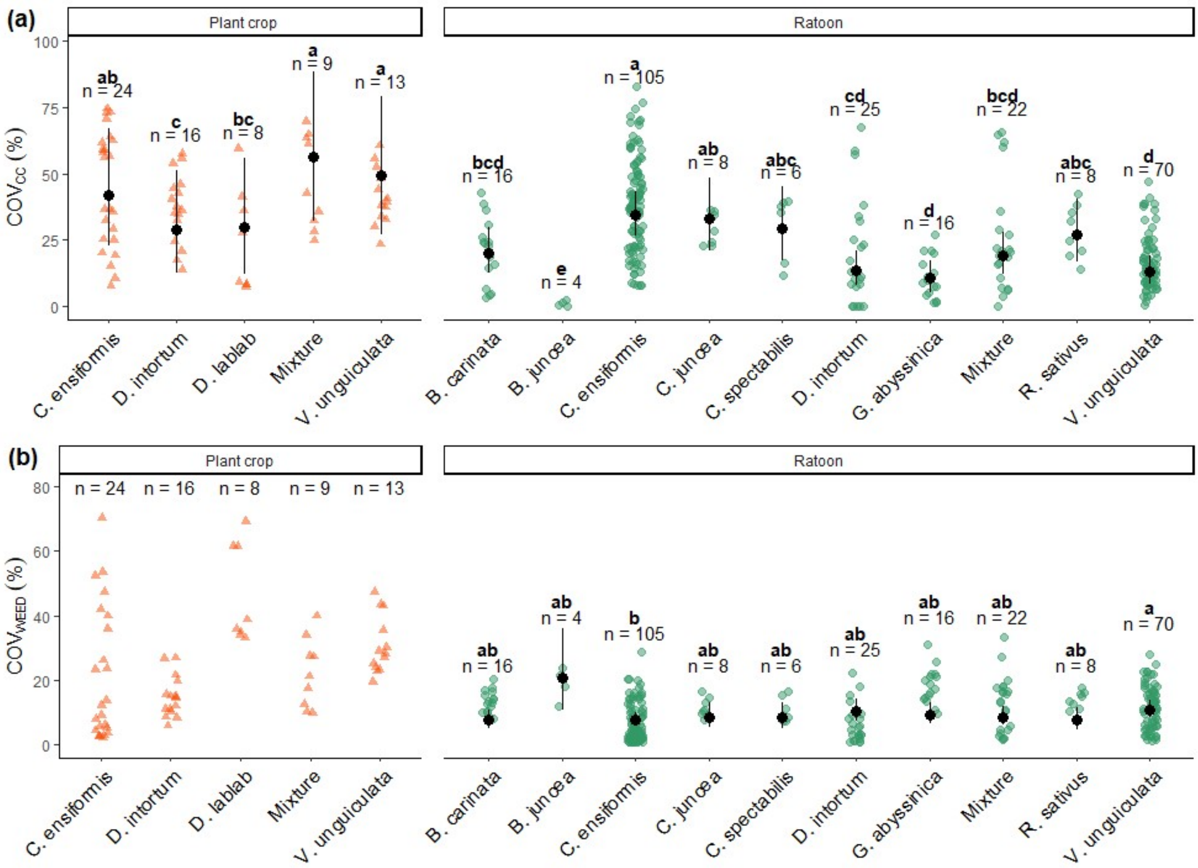

Fig. 5. Mean ground cover by cover crop (COV_{CC}, a) and weeds (COV_{WEED}, b) in the cover crop treatment depending on the cover crop species in plant and ratoon crops. The mean values (black point) and confidence intervals (black line) predicted by the SEM are presented depending on cover crop species. The number of measurements in each category is indicated by "n". Letters indicated significant differences in the pairwise comparison with a Tukey p-adjustment method.

3.5. Weeds response to cover crop management

The structural equation model showed a direct effect of cover crop sowing date on COV_{WEED} in plant and ratoon crops (Fig. 3, 6a) and a direct effect of the number of years of intercropping on COV_{WEED} (Fig. 3, 4b). COV_{CC} was also influenced by the sowing date but only in plant crops (Fig. 3, 6b). In plant crops, the impact of the sowing date on COV_{WEED} was higher than in ratoon (see coefficient in Fig. 3c,d). COV_{WEED} decreased in plant crops by around 5% per month after sugarcane planting and by around 2% in ratoon (Fig. 5a). Similarly, COV_{CC} decreased by around 13% per sowing month after planting in plant crops (Fig. 5b). COV_{WEED} also increased with the number of years since the implementation of intercropping by around 3% per years (Fig. 4b).

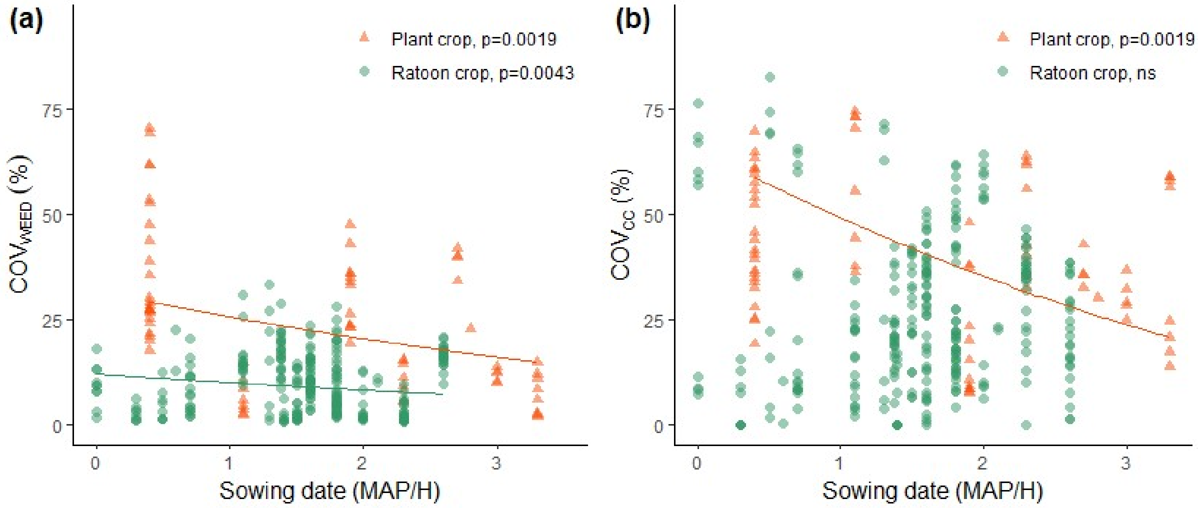

Fig. 6. Mean ground cover by weeds (COV_{WEED}, a) and cover crop (COV_{CC}, b) in the cover crop treatment depending on the sowing date of the cover crop (in months after sugarcane planting or harvest, MAP/H). The lines represented the predicted response by the SEM models after back-transformation, and the P values are indicated.

4. Discussion

4.1. Weed control by cover crops in sugarcane intercropping systems

In our cover crop treatments, weed control was performed by combining preliminary herbicide application (whole plot), cover crop development, and manual weedings (particularly for the weeds considered harmful, tall grasses). Nonetheless, our approach highlights the prominent role of cover crop development in reducing weed infestation in our tropical conditions at the same level as chemical treatments.

Despite having a limited number of studies in the sugarcane literature examining the effectiveness of cover crops for inter-row weed management, these findings agree with previous studies showing that cover crops showed weed suppression efficiency higher than 30% in India (Geetha et al., 2019), and that sugarcane intercropping allow to limit weed biomass at the same level than chemical treatments (Kaur et al., 2016, 2015; Mahadevaswamy and Martin, 2002). Similar observations were made in other intercropping systems in temperate climatic conditions, with a decrease in weed biomass by 56% compared to non-weeded cropping systems in a meta-analysis on various cash crops (mainly wheat, maize, and barley, Verret et al., 2017).

The negative relationship between the weeds and cover crop ground covers observed in our study highlights how the ability of cover crops to limit weed growth is directly linked to its development in the inter-row. These results agree with the broad agreement on cover crop fallow or intercropping that high biomass, cover, or a fast ground cover by cover crops increases weed control (Gardarin et al., 2022; Osipitan et al., 2019). Even if only the mean ground cover by cover crops was tested in this study, the main species used in intercropping are known to have high biomass and rate of increase in ground cover in Reunion Island (Christina et al., 2021b; Négrier et al., 2023).

While the choice of cover crop species appeared important regarding cover crop development in our study, the impact on weed growth was relatively constant. Such a statement on weed growth can result from the impact of complementary manual weedings, even if we did not see a link between MW_{IR} and COV_{WEED} in our SEM approach. Both legumes (*C. ensiformis*, *Crotalaria* species) and non-legume species (*R. sativus*) showed high covering ability. In particular, *C. ensiformis* have a high growth rate and biomass production, which can explain its smothering effect (Lawson et al., 2006). Soares et al. (2022) also showed that *C. ensiformis* reduced the diversity of weed species. *C. juncea* has a potential allelopathic effect that can suppress weeds and the main crops (Skinner et al., 2012). The weed-smothering efficiency of sunn hemp (*C. juncea*) and cowpea (*V. unguiculata*) was also observed in previous studies in India (Geetha et al., 2019). *B. carinata* and the mixtures of cover crops were also adapted to limit weed growth. Nonetheless, other species, such as *D. intortum* or *G. abyssinica*, were less adapted to intercropping.

4.2. Climate, resource, and weed control in intercropping systems

In our climatic conditions, rainfall and temperature influenced weed development. Such a response was expected in our tropical conditions (Peters et al., 2014). Nonetheless, while

COVWEED increased in the low control and chemical treatments with rainfall, it did not change in the cover crop treatments. These results suggest that other factors limit weed development.

In fertilized tropical conditions, one hypothesis is that light partitioning is a primary factor determining the ability of weeds/cover crops to compete (Christina et al., 2021b; Tardy et al., 2015). Light competition can explain the differences in weed cover observed in our study in response to cover crop ground cover and between the plant (slow sugarcane canopy closure, around six months after planting) and ratoon crops (fast sugarcane canopy closure, around 3-4 months after harvest), even if a strong effect of mulch should also be expected (Carvalho et al., 2017). The plant or ratoon crop cycle also influenced cover crop growth in our study, illustrating the importance of light competition between sugarcane and cover crops (Viaud et al., 2023). Previous studies have also shown that cover crops limit the emergence of weeds due to a decrease in the quantity and quality of light transmitted through the cover (Benvenuti et al., 2001; Juroszek and Gerhards, 2004). Accordingly, the cover crop sowing date strongly influenced crop development in plant crops in our study compared to ratoon crops, where the sugarcane dominancy limits cover crop growth. Nonetheless, as suggested by our SEM results, the response of weeds to cover crop sowing dates is more complex. In our study, when the cover crop sowing date was delayed, chemical weeding was performed before sowing, thus decreasing the mean weed ground cover over the crop cycle (direct effect). An opposite effect also occurred; mean cover crop cover decreased by delaying the sowing date (and so increased weed ground cover over the crop cycle, indirect effect).

Our spatially limited study did not allow us to assess the influence of soil characteristics on the weed-cover crop competition in sugarcane intercropping systems. Nonetheless, Viaud et al. (2023) showed a higher impact of intercropping on sugarcane yield with lower soil carbon content in our tropical conditions, suggesting that soil nutrient competition could occur despite fertilization. The influence of soil resource availability on the weeds x cover crops x main crop competition process has to be further investigated as it is still poorly understood and often limited to nitrogen (Corre-Hellou et al., 2011; Hauggaard-Nielsen et al., 2001). Crop modeling could give helpful insights into understanding how and when competition for resources occurred between plants over the sugarcane growth cycle and also help disentangle the effects of these competitions regarding climate or crop management effects.

4.3. Sugarcane intercropping management.

As shown in our study, the combination of cover crops and manual weedings makes it possible to reduce by 37% the frequency of herbicide application in sugarcane cropping systems. However, intercropped species must be carefully selected and their management adapted because of the risk of excessive inter-specific competition on sugarcane yield depending on soil and climatic conditions (Viaud et al., 2023).

Considering cover crop species selection and management, our study suggests that many species would be adapted to sugarcane intercropping regarding weed control. Nonetheless, irrigation failure can affect cover crop emergence and development, highlighting the need to select drought-resistant species such as *C. ensiformis*. Some species, such as *C. juncea*, can also be infested by aphids limiting their development. Cover crop mixtures could help maintain ground cover in case of choosing one sub-optimal species (Négrier et al., 2023) and increase mixture resilience to unexpected events (Elhakeem et al., 2021). Regarding sowing management, our study suggests that early sowing will provide higher cover crop development in plant crops. However, a meta-analysis suggested that early sowing will also induce a higher sugarcane yield reduction in intercropping compared to conventional management (Viaud et al., 2023). Our analysis did not allow us to accurately compare cover crop sowing management, such as the number of sown lines or the sowing method (manual vs. mechanical). Nonetheless, cover crop sowing geometry should be assessed further as previous studies have shown that it can increase cover crop growth with little impact on sugarcane yield (Nadeem et al., 2020; Tian et al., 2020).

While using cover crops limits weed growth in the inter-row, weeding sugarcane row remains a major issue. Herbicides can be sprayed in the sugarcane row to control weeds but with a high risk of affecting cover crop development. Consequently, to limit weeds growth in the row and very harmful weed species in the inter-row, more manual weedings were performed in the sugarcane row and inter-row compared to chemical control. Such interventions could decrease the profitability of intercropping. Despite this effect and the yield response, some studies have shown that additional returns from the intercrop could increase the net profit compared to sugarcane monocropping (Kaur et al., 2015; Nazir et al., 2002; Singh et al., 2008). Nonetheless, such a change in profitability will be highly variable depending on socioeconomic conditions.

In opposition to the long-term effect of legume intercropping on soil fertility (Luo et al., 2016), the long-term effect of intercropping on weed pressure has not been investigated in sugarcane intercropping systems. Our results highlighted how the increase in weed cover with years of intercropping questioned the long-term sustainability of this practice. Indeed, they can explain the previous results of increased sugarcane yield loss through years in sugarcane-legume intercropping systems compared to monocropping (Viaud et al., 2023). The authors' hypothesis to explain such an impact was an increasing weed pressure over time. This increase could result from a weed seed bank enrichment or the selection of highly competitive weeds, as seen in other temperate cover cropping systems mainly under rotations (Graziani et al., 2012; Mohler et al., 2018). Nonetheless, while some studies attempted to assess the response in weed communities in short-term intercropping (Gomez and Gurevitch, 1998; Poggio, 2005; Stefan et al., 2021), the long-term impact of intercropping on change in weed communities still need to be assessed.

Conclusions

Sugarcane intercropping in Reunion Island allowed reducing weed cover by 51% compared to low control treatments and reduced herbicide treatment frequency index by 37% compared to chemical treatments, at the cost of an increased number of manual weedings. In intercropping, crop management significantly influences weed cover more than the climate in our tropical conditions. The lack of a relationship between manual weedings and weed cover suggests investigating the effects of further reduction of costly manual weedings in future research. Additionally, the increase in weed cover with years of intercropping highlights the need to assess the long-term impact of intercropping on weed communities to assess this practice’s sustainability.

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Author contributions

Mathilde Soulé: Investigation, Data Duration, Writing - Original Draft, Vizualisation. **Alizé Mansuy**: Conceptualization, Methodology, Investigation, Project administration, Funding acquisition. **Julien Chetty**: Conceptualization, Methodology, Investigation. **Sandrine Auzoux**: Data Curation, Visualization. **Pauline Viaud**: Formal analysis, Data Curation, Visualization. **Marion Schwartz & Aude Ripoche**: Resources, Visualization. **Benjamin Heuclin:** Formal analysis. **Mathias Christina**: Methodology, Formal analysis, Data Curation, Writing - Original Draft, Visualization, Supervision. **All authors**: Writing - Review & Editing.

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Communications auprès des partenaires.

Ci-dessous sont fournis les communications par affiches issues de la thèse lors d'évènements d'échanges avec les partenaires de la filière canne à La Réunion (disponible en libre accès sur les liens).

Viaud Pauline, Horeau J., Christina Mathias, Versini Antoine, Mansuy Alizé, Marnotte Pascal. 2019. Association canne / légumineuse pour augmenter la fertilité des sols. . DPP-SIAAM. Saint-Gilles : DPP-SIAAM, 1 p. Comité scientifique et technique du DPP SIAAM, Saint-Gilles, Réunion, 5 Novembre 2019/5 Novembre 2019. https://agritrop.cirad.fr/600667/

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Association canne / légumineuse pour augmenter la fertilité des sols

P Viaud[1], J Horeau[1], M Christina[1], A Versini[2], A Mansuy[3], P Marnotte[1]

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Dispositif SALSA : Association canne / pois de sabre blanc

3 modalités :

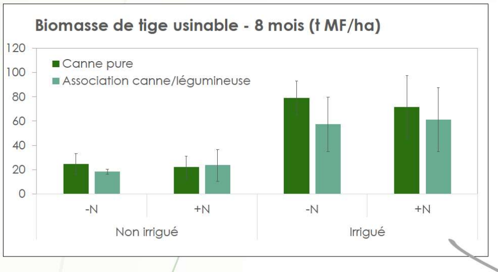
- Fertilisation azotée
- Irrigation
- Pois de sabre (*Canavalia ensiformis*) en intercalaire

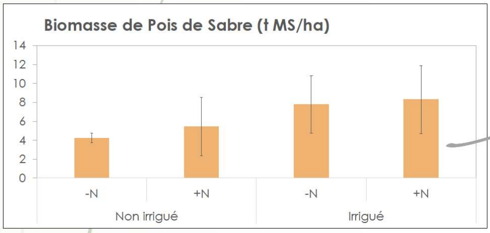
Plantation en Octobre 2018
Site : La Mare – Réunion

Objectifs :

Évaluer la capacité des légumineuses à augmenter la fertilité des sols et l'efficience d'utilisation des ressources, en association avec la canne à sucre.

Résultats préliminaires : Bilan azoté positif





- Le manque d'eau entraine une baisse importante de la production.
- L'absence de fertilisation azotée n'a pas d'effet sur la biomasse de tige à 8 mois par contre il y a une baisse de la production à la coupe pour les traitements sans urée (données non présentées).
- Baisse de rendement pour les modalités avec PDS en intercalaire → compétition importante pour les ressources à la plantation.

− **90 kg N/ha** : tige usinable exportée
(Production Tige 2019 – modalité +N,+W : 131 t MF/ha)

+ **77 à 128 kg N/ha** : fixation symbiotique
(Production PDS 2019 – modalité +N,+W : 8 t MS/ha)

= **Maintien de la fertilité du sol**
Supplément d'azote pour le cycle suivant

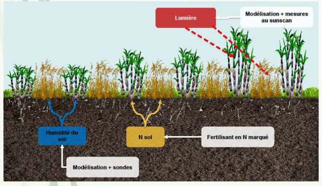
Perspectives : Evaluation multi-services des systèmes canne/légumineuses

Thèse Pauline Viaud (2019-2022)

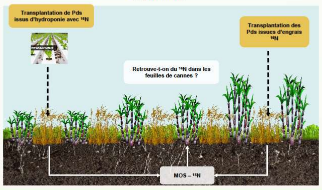
- Etudier les processus de compétition pour les ressources (azote, eau, lumière) et de facilitation (fixation symbiotique et restitution de l'azote)
- Quantifier la réduction potentielle d'engrais minéral à moyen terme
- Paramétrer le modèle STICS-CA pour évaluer les systèmes canne/légumineuses *(rendement, fertilité des sols, efficience d'utilisation des ressources, contrôle des adventices, rentabilité des cultures)*

→ Réseau Dephy Expé et Ecocanne

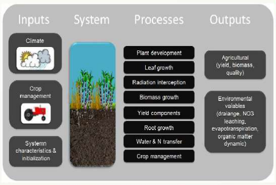
Compétition pour les ressources



Facilitation : transfert N vers la canne



Simulation efficience d'utilisation des ressources



cirad
LA RECHERCHE AGRONOMIQUE POUR LE DÉVELOPPEMENT
1962 2022 60 ANS À LA RÉUNION

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Réponse des rendements cannier à l'association avec des légumineuses, une méta-analyse

Interactions canne/légumineuse, une alliance complexe

- Quel est l'impact des légumineuses en inter-rang sur les rendements de la canne ?
- Quels sont les conditions & itinéraires techniques optimaux ?

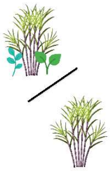
Méthodologie

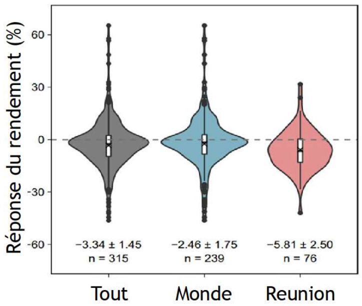
10 ans d'essais à La Réunion
eRcane/Cirad
+
31 études sélectionnées dans le monde
(9 pays)

$$\frac{\text{Rendement}_{\text{CAS+LEG}}}{\text{Rendement}_{\text{CAS}}}$$

Diversité de contextes pédo-climatiques & ITK

Résultat principal





Dispositif experimental eRcane 2022 La Mare
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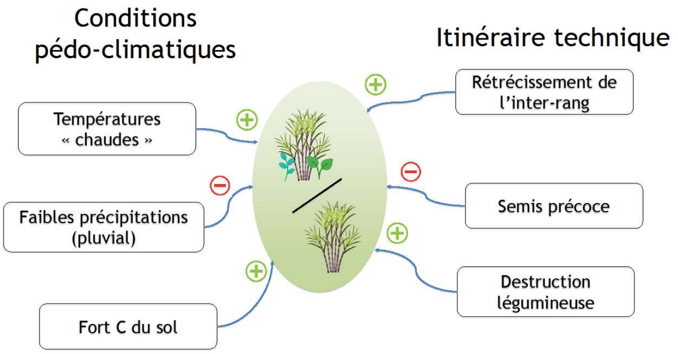
Conditions favorisant les rendements



Conditions pédo-climatiques

Températures « chaudes »

Faibles précipitations (pluvial)

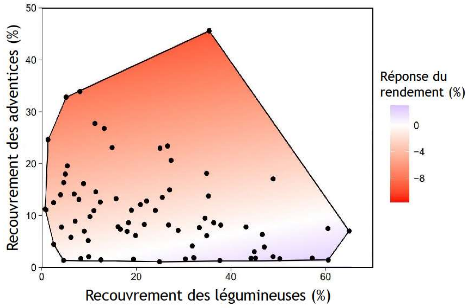
Fort C du sol

Itinéraire technique

Rétrécissement de l'inter-rang

Semis précoce

Destruction légumineuse



À RETENIR !

- ✓ Une réduction de 6% des rendements en moyenne à La Réunion mais très variable
- ✓ Des pistes d'itinéraire technique pour limiter l'impact
- ✓ Les adventices sont deux fois plus nuisibles que les légumineuses dans l'inter-rang

UNION EUROPÉENNE
l'Europe s'engage à La Réunion
PRÉFET DE LA RÉGION RÉUNION
Liberté Égalité Fraternité
RÉGION RÉUNION
www.regionreunion.com
RITA CANNE RÉUNION
Réseau d'innovation et de transfert agricole
DÉPARTEMENT DE LA Réunion
Valoriser la ressource canne
eRcane

SYSTÈMES DE CULTURES INNOVANTS

Les légumineuses exercent-t-elles une pression compétitive sur la canne-à-sucre ?

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UPR 115 AIDA, Saint-Denis, La Réunion
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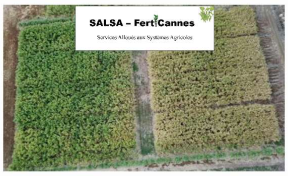
Dispositif SALSA : Association canne-à-sucre x *Canavalia ensiformis*

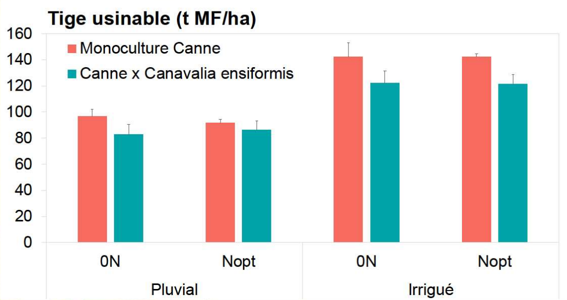
3 facteurs :
- Irriguée ou pluviale
- Fertilisation azotée : 0N / Nopt
- Canne pure ou Canne x *Canavalia ensiformis*

Plantation en Octobre 2018
Site : La Mare – Réunion



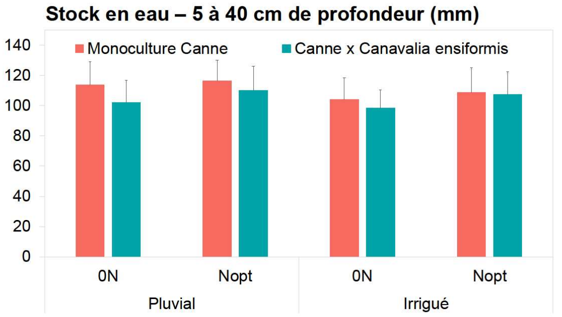
Objectifs :

Étudier la répartition des ressources (rayonnement, eau et azote) entre les deux espèces et le devenir de l'azote dans l'agrosystème.



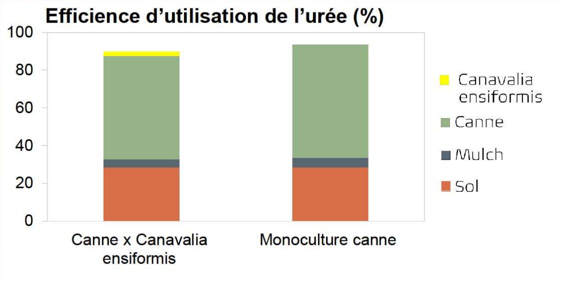
Effet sur le rendement en canne :

- Le manque d'eau entraine une baisse importante de production.
- L'absence de fertilisation azotée n'a pas d'impact sur la production.
- Les légumineuses induisent une baisse de rendement de 10 % en système pluvial et 15 % en système irrigué.



Compétition pour l'eau :

Les stocks en eau sont en moyenne 12% (pluvial) et 6% (irrigué) plus faibles en présence de légumineuses (période de cohabitation).



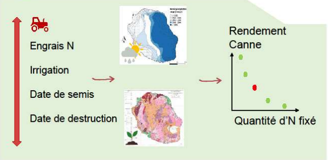
Compétition pour l'N du fertilisant :

- La canne utilise 55 % de l'urée apportée.
- La quantité prélevée par les légumineuses est négligeable.

Perspectives : Modélisation des systèmes canne-à-sucre x légumineuses

Optimiser les systèmes canne-à-sucre x légumineuses en fonction des conditions pédoclimatiques de La Réunion via des expérimentations virtuelles de stratégies de gestion :

- Date de semis et destruction de la légumineuses
- Quantité d'engrais apportée
- Irrigation



Contact : *pauline.viaud@cirad.fr*