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Nitrogen fluxes in agroforestry : experiments and modelling approaches

Mubarak Mahmud

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Mubarak Mahmud. Nitrogen fluxes in agroforestry : experiments and modelling approaches. Life Sciences [q-bio]. Université Paris-Saclay, 2023. English. NNT : 2023UPASB074 . tel-04417489

HAL Id: tel-04417489

<https://hal.science/tel-04417489v1>

Submitted on 2 Jan 2025

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Nitrogen fluxes in agroforestry: experiments and modelling approaches

Flux d'azote en agroforesterie : expérimentation et modélisation

Thèse de doctorat de l'université Paris-Saclay

École doctorale n° 567 sciences du végétal : du gène à l'écosystème (SEVE),
Spécialité de doctorat : Ecologie
Graduate School : BIOSPHERA. Référent : Faculté des sciences d'Orsay

Thèse préparée dans l'unité de recherche **Ecologie Systématique et Evolution**
(Université Paris-Saclay, CNRS, AgroParisTech), sous la direction de
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Titre : Flux d'azote en agroforesterie : experimentation et modélisation

Mots clés : azote, agroforesterie, ^{15}N , Hi-sAFe, modélisation, CO_2 élevé

Résumé : Dans le domaine de l'agriculture durable et de l'adaptation au climat, l'agroforesterie s'est imposée comme une solution prometteuse. Cette thèse de doctorat examine l'agroforesterie méditerranéenne impliquant des robiniers, en mettant l'accent sur la dynamique de l'azote et la réponse du système agroforestier au changement climatique et à l'augmentation du CO_2 . Le contexte général de l'étude était de tester l'hypothèse du filet de sécurité des éléments nutritifs en agroforesterie, dans laquelle les arbres et la végétation du sous-étage sont supposés aider à éviter les pertes d'azote dans l'environnement par lessivage des nitrates vers la nappe phréatique. Cela a été réalisé en utilisant le marquage au ^{15}N dans un système expérimental et en suivant le devenir du ^{15}N sur deux ans dans les arbres, les sols et les cultures. Les impacts du changement climatique et de l'augmentation du CO_2 ont été explorés à l'aide d'un modèle agroforestier mécaniste, Hi-sAFe, dans lequel les effets connus et importants d'une augmentation du CO_2 sur les arbres ont été nouvellement introduits dans le modèle. Les hypothèses de recherche initiales suggéraient que les arbres, la bande de végétation du sous-étage (UVS) et la biomasse microbienne du sol (BM) absorberaient le ^{15}N , réduisant ainsi le lessivage des nitrates. Contrairement à ces hypothèses, la première année de l'étude n'a fourni aucune preuve de ^{15}N ni dans les arbres, ni dans les UVS, ni dans le BM. Cependant, en 2022 (la deuxième année), des preuves d'absorption du ^{15}N sont apparues. Il a été démontré que les arbres absorbaient environ 2 % du ^{15}N appliqué. L'estimation du stock d'azote a démontré le potentiel plus important de l'agroforesterie (AF) que de la monoculture (MC), avec une teneur relative en azote (RNC) étant de 0,97 en 2021 et de 1,63 en 2022. En plus de la dynamique de l'azote, nous avons également émis l'hypothèse que le rendement des cultures serait plus élevé. être considérablement réduit en AF par rapport au MC. Cette hypothèse a été confirmée dès la première année, où AF a donné un rendement d'environ 20 % inférieur à MC. L'étude a en outre estimé le pourcentage d'azote dérivé de l'atmosphère (Ndfa) dans les robiniers, obtenant des valeurs allant de 52 % à 68 %. Au cours de la dernière année de recherche, l'accent a été mis sur la modélisation des effets d'une concentration élevée de CO_2 sur les noyers hybrides en agroforesterie. Ceci a été réalisé en introduisant les effets du CO_2 dans le modèle Hi-sAFe. Les résultats ont montré que la hauteur des arbres réagit positivement à l'augmentation du CO_2 et au changement climatique, mais qu'il n'y a pas d'effet significatif sur le diamètre à hauteur d'homme. De plus, le lessivage des nitrates a été réduit en cas de CO_2 élevé. La recherche a des implications pratiques pour la gestion des éléments nutritifs en agroforesterie, où les arbres et les bandes de végétation du sous-étage peuvent retenir l'azote lessivable, améliorer les stocks d'azote au niveau du système, ce qui pourrait contribuer à la fertilité des sols et à l'agriculture durable. L'amélioration des modèles écologiques tels que Hi-sAFe peut fournir des informations plus précises sur l'adaptation climatique dans l'agroforesterie et la croissance des arbres dans des conditions élevées de CO_2 . La thèse de doctorat élargit les connaissances existantes en examinant les contributions des jeunes arbres, en mettant l'accent sur les variations de profondeur dans les analyses de sol et en améliorant les modèles d'évaluation des effets du CO_2 . La recherche s'appuie sur une gamme d'atouts, notamment des expériences bien structurées, des tests d'hypothèses, l'utilisation du marquage isotopique et une collaboration interdisciplinaire. Cependant, elle présente également des limites, telles que la nature à court terme de l'étude et la nécessité de recherches plus approfondies sur le rôle des racines du sous-étage et d'études microbiennes et nutritionnelles des sols profonds.

Title : Nitrogen fluxes in agroforestry : experiments and modelling approaches

Keywords : nitrogen, agroforestry, ^{15}N , Hi-sAFe, modelling, elevated CO_2

Abstract : In the aspect of sustainable agriculture and climate adaptation, agroforestry has risen as a promising solution. This PhD thesis examines Mediterranean agroforestry involving black locust trees, with a focus on nitrogen dynamics and the agroforestry system's response to climate change and elevated CO_2 . The overall background of the study were to test the nutrient safety net hypothesis in agroforestry, in which trees and understory vegetation are hypothesized to help avoid nitrogen losses to the environment through nitrate leaching to the water table. This was done by using ^{15}N labelling in an experimental system, and following the fate of ^{15}N over two years in trees, soils and crops. The impacts of climate change and rising CO_2 were explored using a mechanistic agroforestry model, Hi-sAFe, where the known and important effects of elevated CO_2 on trees were newly introduced into the model. The initial research hypotheses suggested that trees, Understory Vegetation Strip (UVS) and soil microbial biomass (BM) would absorb ^{15}N , thus reducing nitrate leaching. Contrary to these hypotheses, the first year of the study did not provide evidence of ^{15}N in either the trees, UVS or the BM. However, in 2022 (the second year), evidence of ^{15}N absorption emerged. The trees were shown to absorb about 2% of the applied ^{15}N . Estimation of nitrogen stock demonstrated the significant potential of agroforestry (AF) than monocrop (MC), with the Relative Nitrogen Content (RNC) being 0.97 in 2021 and 1.63 in 2022. In addition to the nitrogen dynamics, we also hypothesized that crop yield would be significantly reduced in AF compared to MC. This hypothesis was confirmed in the first year, where AF yielded approximately 20% less than MC. The study further estimated the percentage of nitrogen derived from the atmosphere (NDFA) in black locust trees, obtaining values ranging from 52% to 68%. In the final year of the research, the focus was modeling the effects of elevated CO_2 on hybrid walnut trees in agroforestry. This was accomplished by introducing CO_2 effects into the Hi-sAFe model. The results showed that tree height positively responds to both elevated CO_2 and climate change, but there was no significant effect on diameter at breast height. Additionally, nitrate leaching was reduced under elevated CO_2 . The research carries practical implications for nutrient management in agroforestry, where trees and understory vegetation strips can retain leachable nitrogen, enhance nitrogen stock at the system level, which might contribute to soil fertility and sustainable agriculture. Refining ecological models like Hi-sAFe can provide more accurate insights into climate adaptation in agroforestry and tree growth under elevated CO_2 . The PhD thesis expands the existing knowledge by examining young tree contributions, emphasizing depth variations in soil analyses, and improving models for CO_2 effects assesment. The research employs a range of strengths, including well-structured experiments, hypothesis testing, the use of isotope labeling, and interdisciplinary collaboration. However, it also has its limitations, such as the short-term nature of the study and the need for further research into the role of understory roots and deep soil microbial and nutrient studies.

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ACKNOWLEDGEMENT

First and foremost, I am immensely grateful to the CLAND Convergence Institute, France (ANR-16-CONV-0003) and the Kano State Government of Nigeria for their generous funding that made my research and education possible. I would like to extend my heartfelt appreciation to Louise Darmancourt, John Bazir, and Dr. Ali Haruna Makoda for their unwavering support. I am indebted to DIAMS, Montpellier, and Isabelle Bertrand for providing me with the experimental site for my research.

I am deeply grateful to my research supervisors, Laure Barthes, Paul Leadley, Nicolas Delpierre, and Stephane Bazot, for their guidance and mentorship. Your insights and expertise were invaluable in shaping my study. And to my research collaborators, Isabelle Bertrand, Pascale Maillard (from my thesis committee), Christian Dupraz, Marie Gosme, Isabelle Lecompte, Nicolas Barbault, and Francois Decoligny. A sincere thank you goes to the researchers, engineers and technicians who provided scientific and research support, including Jérôme Ngao, Claire Marsden, Remi Dugue, Marion Forest (from UMR Eco&Sols), Alexandre Morfin, Gaëlle Vincent, Alain Severe (from ESE laboratory) and Anne Marmagne (from INRAE Versailles). I also want to appreciate the administrative support team: Nathalie Lecat, Sandrine Dessaints, and Nadia Livet.

I want to express my gratitude to my father, Alh Mahmud Kabir, my mother, Haj. Rabiātu Tahir, my beloved grandmother, Late Fatima Tahir, and my mentor and brother, Engr Faisal Mahmud. My family Haj. Rukayya Usman (Umma), Haj. Amina (Gwaggo), Engr Abubakar Abdullahi (Yaya Alhaji), Tahir and Faruk Mahmud, Mulaikha, Fatima and Safiyya Mahmud as well as Anty Zainab Sale. My lovely wife, Maryam Aminu Ahmad, I deeply appreciate your prayers and support. And to all my siblings, nephews, nieces, cousins and friends, I love you all.

Prof Gausu Ahmad (my father and mentor), Prof Aminu U. Fagge, Prof Yusuf B. Daraja, Dr Binta M. Aminu (Biology, BUK), Prof Ahmad Usman Marmara, Prof Adamu Abubakar Gwarzo & Sarki Mohamed Sirajou Djankado, your mentorship have had a profound impact on my life and academic journey.

I express my appreciation to my friends, Hicham Tijari, Aminu Wada Fanda, Mustapha Saleh, my namesake Mubarak Mustapha Saleh, Ezzeddine Abbesi, Ibrahim Buba, Sahal, Aude Sany, Christophe François, and Yannick Barthes. Haleema Aminu, thank you for been a source of motivation and inspiration for me.

DEDICATION

This Ph.D. is dedicated In Loving Memory of My Beloved Late Uncle, **Muhammad Awwal Tahir**.

To my dearest late uncle, Muhammad Awwal Tahir, whose presence in my life was a blessing beyond measure. Though you are no longer with us in the physical sense, your memory and the profound impact you had on my life remain etched in my heart.

You were a guiding light, a source of wisdom, and a pillar of strength. Your kindness, your unwavering support, and your enduring love were a constant source of inspiration for me.

Your memory continues to be a source of comfort and solace, and your legacy lives on through the values and principles you instilled in me. I will forever cherish the moments we shared, the lessons you imparted, and the love you gave.

Though you may be gone from this world, you will never be forgotten. Your spirit lives on in the hearts of all those who were fortunate enough to know you. You will always be my beloved late uncle, and your memory will forever be a source of inspiration and strength in my life.

Rest in peace. You are deeply missed, and you will always be loved and remembered.

Yours,
Mubarak

Dans la poursuite de pratiques agricoles durables alignées sur l'adaptation au climat, l'agroforesterie est devenue un domaine extrêmement prometteur. Cette thèse de doctorat complète se lance dans un examen détaillé de l'agroforesterie méditerranéenne, avec un accent particulier sur l'implication du robinier. L'objectif principal à travers lequel se déroule cette enquête est l'interaction complexe de la dynamique de l'azote au sein du système agroforestier, associée à une exploration de sa réactivité au changement climatique et à l'augmentation du CO₂. Le contexte général de cette étude est enraciné dans la quête de validation de l'hypothèse du filet de sécurité nutritionnelle inhérente à l'agroforesterie. Selon cette hypothèse, les arbres et la végétation du sous-étage jouent un rôle essentiel dans l'atténuation des pertes d'azote, notamment en évitant le lessivage des nitrates vers la nappe phréatique. Cette exploration est facilitée par l'utilisation stratégique du marquage ¹⁵N dans un cadre expérimental soigneusement conçu, permettant le suivi du devenir du ¹⁵N sur une période exhaustive de deux ans dans les arbres, les sols et les cultures. Parallèlement à l'examen minutieux de la dynamique de l'azote, l'étude se penche sur les répercussions du changement climatique et de l'augmentation du CO₂, en utilisant le modèle agroforestier mécaniste Hi-sAFe. Ce modèle, renforcé par les effets nouvellement intégrés d'une augmentation du CO₂ sur les arbres, sert d'outil sophistiqué pour démêler les réponses nuancées du système agroforestier à ces changements environnementaux. Contrairement aux conjectures initiales, l'année préliminaire de l'étude n'a pas fourni de preuve d'absorption du ¹⁵N par les arbres, les bandes de végétation du sous-étage (UVS) ou la biomasse microbienne du sol (BM). Cependant, le récit prend un tournant l'année suivante, révélant une émergence perceptible de preuves relatives à l'absorption du ¹⁵N. Il a été démontré que les arbres, en particulier, absorbent environ 2 % du ¹⁵N appliqué, ce qui donne du crédit à l'hypothèse du filet de sécurité des éléments nutritifs. En élargissant le champ d'enquête, les estimations des stocks d'azote passent au premier plan, révélant le potentiel substantiel inhérent à l'agroforesterie (AF) lorsqu'elle est juxtaposée aux systèmes de monoculture (MC). La teneur relative en azote (RNC), une mesure clé, présente une augmentation notable de 0,97 en 2021 à 1,63 en 2022, soulignant les avantages de l'agroforesterie au niveau du système. Au-delà du domaine complexe de la dynamique de l'azote, la recherche s'aventure à prédire une réduction significative du rendement des cultures dans le domaine de l'agroforesterie par rapport à ses homologues en monoculture. Confirmant cette hypothèse, la première année voit l'AF produire environ 20 % de moins que son homologue en monoculture. L'exploration ne s'arrête pas là ; elle s'étend à l'estimation du pourcentage d'azote dérivé de l'atmosphère (Ndfa) dans les robiniers, révélant une fourchette allant de 52 % à 68 %. Alors que le récit de recherche entre dans sa phase finale, l'accent se tourne vers la modélisation des effets d'une concentration élevée de CO₂ sur les noyers hybrides dans le contexte agroforestier. Cette modélisation complexe, facilitée par le modèle Hi-sAFe, dévoile une dynamique intrigante dans laquelle la hauteur des arbres réagit positivement à une augmentation du CO₂ et au changement climatique, bien que sans effet

significatif sur le diamètre à hauteur de poitrine. Il convient également de noter la réduction observée du lessivage des nitrates dans des conditions élevées de CO₂. Cet effort de recherche aux multiples facettes s'étend au-delà des domaines de l'exploration théorique pour avoir des implications pratiques pour la gestion des éléments nutritifs dans les systèmes agroforestiers. Le rôle des arbres et des bandes de végétation du sous-étage dans la rétention de l'azote lessivable apparaît comme la pierre angulaire de l'amélioration des stocks d'azote et contribue à la fois à la fertilité des sols et au cadre plus large de l'agriculture durable. La recherche souligne la nécessité d'affiner les modèles écologiques, tels que Hi-sAF_e, pour fournir des informations plus précises sur les complexités de l'adaptation climatique au sein des systèmes agroforestiers. Reconnaisant ses atouts en matière de conceptions expérimentales bien structurées, de tests d'hypothèses, de marquage isotopique et de collaboration interdisciplinaire, l'étude reste consciente de ses limites. La nature intrinsèquement à court terme de l'étude et la nécessité d'une exploration plus approfondie du rôle des racines du sous-étage, ainsi que d'études microbiennes et nutritionnelles des sols profonds, apparaissent comme des pistes pour de futurs efforts de recherche. Essentiellement, cette thèse de doctorat élargit non seulement la frontière des connaissances existantes, mais ouvre également la voie à de futures recherches approfondies sur les complexités des systèmes agroforestiers dans le contexte du changement climatique et de l'agriculture durable.

1 CHAPTER 1 : INTRODUCTION

In the aspect of sustainable agriculture and climate adaptation, agroforestry has risen as a promising solution. As part of my research into the dynamics of nitrogen fluxes and soil-plant interactions in agroforestry systems, this introduction gives a deeper understanding of these complex relationships. I began by defining and classifying agroforestry systems. Next, I discussed the numerous benefits of agroforestry, including enhanced agronomic productivity and soil carbon sequestration. I also examined the vital role of soil biodiversity and soil erosion control in these systems. Additionally, I investigated the potential of herbaceous strips within agroforestry and the issue of nitrogen leaching, followed by a discussion of management strategies to mitigate this problem. This introduction explores the concept of the nutrient safety net and its significance in nutrient retention as well as the use of tracers and models for assessing nitrate leaching, followed by an examination of agroforestry's role in climate change adaptation. Finally, the introduction outlines the research questions that guided this study, emphasizing its contribution to the understanding of nitrogen fluxes within agroforestry systems.

1.1 DEFINITIONS AND CLASSIFICATIONS OF AGROFORESTRY

Agroforestry is a broad concept that embodies a rich diversity of practices and systems, each offering its unique benefits and challenges. By clearly defining and categorizing these diverse forms of agroforestry, we lay the groundwork for understanding their underlying principles, environmental and socioeconomic implications. Agroforestry definition and classification not only provides a systematic framework for our exploration but also highlights the complex relationships between trees, crops, and livestock within the system.

1.1.1 Definitions of agroforestry

The conventional definition of agroforestry is rooted in the concept of cultivating trees or other woody perennial plants alongside crops or livestock (Torquebiau, 2000). Over time, the definition of agroforestry has evolved to encompass a broader range of topics, including but not limited to social and ecological aspects, alternative land use practices, and resource utilization. In 1982, Lundgren and Raintree, working under the International Council for Research in Agroforestry, provided a comprehensive definition of agroforestry. They described it as a collection of land-use systems and techniques that intentionally involve the cultivation of woody perennials alongside crops and animals, either through spatial arrangements or temporal sequences. Since agroforestry systems demonstrate ecological and

economic interactions among their various components, Somarriba (1992) further elaborated, defining agroforestry as a form of multiple cropping that entails cultivating at least two plant species, with one being a woody perennial that interacts biologically, while the other may be managed for forage, annual, or perennial crop production.

However, the initial definition of agroforestry was considered insufficient because it led to land use applications that did not fully tap into its potential for mitigating deforestation, addressing land degradation, and alleviating poverty (Leakey, 1996). Consequently, agroforestry was redefined as a natural resource management system rooted in ecological principles, integrating trees into farms and agricultural landscapes to diversify and sustain smallholder production, leading to enhanced social, economic, and environmental benefits (Sanchez, 1995).

In summary, the modern interpretation of agroforestry portrays it as a multifaceted land use system that seeks a harmonious balance between the production of various commodities (such as food, feed, fuel, and fibre) and non-commodities, including environmental conservation, as well as cultural and aesthetic benefits (IAASTD, 2008).

1.1.2 Classifications of agroforestry

Agroforestry can be classified into three major categories, which are 1) Classification based on practices, 2) classification based on type, and 3)

classification based on simplified structure.

1.1.2.1 Classification based on practice

Practical classification of agroforestry was given as six distinct categories by Mosquera-Losada et al., 2008 as follows:

- (i) Components, such as silvoarable, which combines trees with crops; and silvopastoral, which entails the integration of trees, fodder crops, and animals.
- (ii) Predominant land use, such as land exhibiting a mainly silvicultural character with some agricultural purpose for example forest grazing; or predominantly agricultural characteristics with the incorporation of trees, such as parkland.
- (iii) Spatial and temporal structure, such as the scattered oak trees in the dehesas of Spain or Portugal and the parkland systems in the UK. Also the poplar systems found in Italy and France which are occasionally intercropped with annual crops or perennial fodder crops solely during the initial phase of the tree rotation can be put under this classification.
- (iv) Agroecological zones, such as systems in the tropical and sub-tropical regions that are classified based on their occurrence in humid, sub-humid areas, dry regions, lowlands,

or highlands (Nair, 1985; Young, 1997). A classic example of this classification is the Mediterranean agroforestry system.

- (v) Socio-economic status: This is a classification that includes factors such as production scale, input, and management levels (Sereke et al., 2014). In other words, this is a classification based on commercial or subsistence systems.
- (vi) Function: This is based on the ecosystem's primary functions which can be production (such as biomass), habitat (ecosystem), regulation (biogeochemical and biosphere mechanisms), or information functions (such as cultural and aesthetics) (see Nair et al., 2021).

1.1.2.2 Classification based on Type

There are five fundamental categories of agroforestry in temperate regions: *alley cropping, forest farming, riparian buffer strips, silvopasture, and windbreaks*. However, that classification was revised by Mosquera-Losada et al. (2012) to include *silvoarable agroforestry, forest farming, riparian buffer strips, silvopasture, improved fallow, and multipurpose trees*. See Quinkenstein et al., 2009.

1.1.2.3 Classification based on a simplified structure

To some scholars, all the classifications above of agroforestry could be less precise and more limiting (Kass et al., 1993; King, 1979). For instance, the term agro silviculture, which is essentially interchangeable with agroforestry,

encompasses a vast array of practices. Additionally, numerous agroforestry methods involving animals lack any pastoral connotation; they are not necessarily associated with shepherds or herds. Furthermore, potential ambiguities may arise with respect to the aforementioned classifications. For instance, it may be unclear whether the practice of raising cattle in a forested area that has been planted with pasture should be categorized as silvopastoral (where the pasture is regarded as an integral component of the animal production system) or agrosilvopastoralism (where the pasture is treated as a crop).

To counter those ambiguities, Torquebiau (2000) classified agroforestry based on specific structural criteria that can be observed at first glance, i.e., what is the appearance of the field in question? This classification is presented in table 1 below.

Table 1. Classification of agroforestry in six structural categories

Main structure	Sub-type
Crops undercover	Scattered trees in cropland
	Shade trees in plantation crops
	Parklands
	Plantation crop combinations
	Crops in orchards
Agroforests	Agroforestry and home gardens
	Village forest gardens
	Mixed woodlots
	Agroforestry buffer zones
Agroforestry in a linear arrangement	Alley cropping
	Boundary planting
	Live hedges
	Soil conservation hedgerows
	Woody strips
	Windbreaks and shelterbelts
	Roadside planting
	Living fences
	Animal agroforestry
Grazing or browsing in wooded or forested land	
Animal feeding with collected browse	
Sequential agroforestry	Browse banks
	Taungya
Minor agroforestry techniques	Shifting cultivation
	Tree-improved fallows
	Apiculture with trees
	Lac production
	Sericulture
	Tree-based aquaculture

1.2 BENEFITS OF AGROFORESTRY

Beyond the primary goal of understanding nitrogen dynamics in agroforestry, I am also shedding light on the broader positive impacts of agroforestry. This encompasses improvements in soil health, increased biodiversity, carbon capture, and the support of local communities. This not only emphasized the relevance of my work but also its position within the wider context of sustainable agriculture and land management.

1.2.1 Agronomic Productivity

The land equivalent ratio (LER) is the ratio of the land area required for sole cropping to the land area needed for intercropping to achieve equivalent yields under identical management conditions (Mead & Willey, 1980). LER is the standard measure used to evaluate the productivity of agroforestry compared to monoculture (Malezieux et al., 2009). Various studies have reported the presence of increased productivity in agroforestry systems. Lovell et al. (2017) reported the LER of agroforestry systems in Denmark to vary between 1.14 and 1.34, suggesting that the crop and tree yields obtained through agroforestry required a reduced amount of land or resources, such as light, water, and nutrients, ranging from 14% to 34% when compared to monoculture. This presents a considerable benefit and offers opportunities for ecological intensification to enhance productivity while minimizing resource usage. Another study by Xu et al. (2019), where five different agroforestry systems with varied crop, tree, and grass species,

as well as management regimes, were studied, shows that the productivity of agroforestry systems, as measured by LER values, ranged from 1.36 to 2.00. These results and many others, such as Sereke et al. (2014) and Lehmann et al. 2020, suggest that a diverse range of agroforestry systems can yield higher productivity than monocropping.

1.2.2 Soil Carbon Sequestration

Soil represents a significant carbon reservoir, containing around 1550 gigatons (Gt) of soil organic carbon and 950 Gt of soil inorganic carbon (Lal, 2004). Conventional agricultural practices, which result in the depletion of soil organic matter, cause European agricultural soils to function poorly as carbon sinks (Vleeshouwers & Verhagen, 2002; Sleutel et al., 2003; Smith et al., 2005; Page et al., 2020; Xu et al., 2019). However, there is substantial potential for these soils to become effective carbon sinks by implementing changes in land management techniques (Vleeshouwers & Verhagen, 2002; Lal, 2004).

Agroforestry has been found to enhance carbon sequestration through various mechanisms, such as root production, vegetation deposition through litter fall, and carbon accumulation through the woody biomass of trees, which contains approximately 46–51% carbon (Gruenewald et al., 2007; Mosquera-Losada et al., 2011; Lorenz & Lal, 2014; Kim et al., 2016; Carranca et al., 2022). Field experiments have demonstrated that the

presence of trees resulted in a significant increase of 6.2 and 5.3 tons of organic carbon per hectare, respectively, as compared to the control plots without trees (Bambrick et al., 2010; Pardon et al., 2017). The incorporation of woody crops, such as willow, into conventional agriculture has the potential to increase carbon sequestration by up to 103 t ha⁻¹, encompassing aboveground, belowground, and soil organic carbon (Bazrgar et al., 2020). A study conducted to determine critical regions in Europe where the adoption of agroforestry systems could yield significant benefits, including the effects on soil carbon, shows that the adoption of agroforestry has the potential to sequester carbon in the range of 2.1 to 63.9 million t C ha⁻¹ (Kay et al., 2019). This range of carbon sequestration corresponds to 1.4% to 43.4% of the total greenhouse gas emissions produced by European agriculture. According to Aertsens et al. (2013), the adoption of agroforestry practices on arable land within the European Union has the capacity to sequester approximately 2.75 t C ha⁻¹ year⁻¹.

Microbial biomass constitutes the biotic component of soil organic matter and typically accounts for 1–5% of the soil's overall organic carbon content (Verma et al., 2013). Despite constituting a minor fraction of soil carbon, the carbon present in microbial biomass still constitutes a significant global reservoir, estimated to be approximately 23 petagrams (Xu et al., 2013). Thus, soil microorganisms in agroforestry Systems lead to increased carbon sequestration.

1.2.3 Nutrient Cycling

Nutrients in Agroforestry can be derived from pruning and tree litter fall, rendering them accessible for the crops grown in the intercropped alleys (Schneidewind et al., 2019; Rivest et al., 2013). Individual tree biomass in agroforestry systems is comparatively higher than individual trees in forests due to reduced inter-tree competition, which can subsequently be reintegrated into the system, thereby enhancing soil organic matter and nutrient recycling (Van Noordwijk & Purnomosidhi, 1995; Livesley et al., 2000). Additionally, tree roots can access and utilize nutrients that have leached into deep soil layers, beyond the reach of crop roots (Isaac & Borden, 2019).

Studies have confirmed that nutrient availability and cycling are more substantial and effective in agroforestry systems than in monocultures (Gupta et al., 2010; Rivest et al., 2013; Schneidewind et al., 2019). That can be attributed to various mechanisms, such as the inclusion of nitrogen-fixing trees or the adoption of leguminous crops in crop rotation or as cover crops, which result in elevated levels of nitrogen reserves (Rosenstock et al., 2014; Koutika et al., 2021). A study shows a mineralized nitrogen quantity of 108 kg N ha⁻¹ year⁻¹ in agroforestry, higher than the 64 kg N ha⁻¹ year⁻¹ observed in a conventional wheat production system (Ghaley et al., 2014). Furthermore, Pardon et al. (2017) observed higher nutrient concentrations in agroforestry systems compared to control plots without trees. Thevathasan

and Gordon (2004) found that in agroforestry systems, the leaf litter from nearby poplar trees resulted in a twofold increase in the nitrogen availability in soils for crop uptake in the alleys, compared to soils situated at a distance of 8 to 11 meters from the trees.

1.2.4 Soil biodiversity

Microorganisms, including algae, bacteria, fungi, and microarthropods, serve as decomposers and nutrient transformers and are integral parts of organic matter breakdown, which results in nutrients being released and contributes to its cycling (Coleman et al., 2004; Dollinger et al., 2018). The majority of studies that analyzed agroforestry in comparison to arable systems with respect to biodiversity reported favourable outcomes in agroforestry (Torralba et al., 2016; Marsden et al., 2020). Agroforestry has been found to promote the proliferation of arbuscular mycorrhizal fungi, which in turn accelerates the process of litter decomposition and increases plant nutrient availability (Chiffot et al., 2009; Schadler et al., 2010).

The physical characteristics and permeability of soil are modified by ants and earthworms through the creation of burrows, chambers, and castings, thereby increasing the flow of nutrients and energy (Kibblewhite et al., 2007; Cardinael et al., 2018a). Several studies have shown that the abundance and biomass of earthworms and organic carbon stocks were comparatively greater in agroforestry than in control plots (Price and Gordon 1998; Barea

et al., 2005; Ghaley et al., 2018). A greater variety of soil macroorganisms, such as earthworms, beetles, centipedes, millipedes, termites, ants, collembola, mites, and non-parasitic nematodes, has been observed in agroforestry systems (Barrios et al., 2012). Quantification of bacterial 16S ribosomal RNA (rRNA) genes performed on soil samples obtained from agroforestry comprising woodland or hedgerows, as well as control samples from either grassland or agricultural land, indicated a greater abundance of soil bacteria in agroforestry (Banerjee et al., 2015). Fungal richness was also observed to be higher in agroforestry than in monocrop (Bainard et al., 2011).

1.2.5 Soil erosion control

Agroforestry systems have been found to offer numerous benefits, including the reduction of surface runoff and improved infiltration into the soil, which helps recharge aquifers (Udawatta et al., 2002; Udawatta et al., 2010). Agroforestry systems enhance soil stability and mitigate erosion through multiple mechanisms, such as increased ground cover resulting from the presence of leaf litter and "tree mulch" that intercept rainfall, diminish the speed of surface runoff, lower evapotranspiration, and restrict soil crusting (Sepúlveda & Carrillo, 2015). Increased levels of organic matter have been found to enhance the soil's capacity to retain water and promote stability by enhancing soil aggregation and structure (Sepúlveda & Carrillo, 2015). In addition, trees and hedges serve as a permeable barrier that decelerates and

intercepts the flow of water runoff while Windbreaks and shelterbelts are implemented to mitigate wind erosion (Mutegi et al., 2008). Additionally, tree mulches, cover crops, and alley cropping techniques have been found to effectively mitigate soil temperature and soil erosion (Lin, 2010).

Alley cropping has been found to decrease soil loss by as much as 80% (Sajjapongse et al., 2002). Budelman (1989) demonstrated that the utilization of mulches derived from trees or woody shrubs could lead to a reduction in soil temperature of up to 6.6°C and an increase in soil moisture content of up to 4.6%. Shading has been identified as a regulatory mechanism for soil evaporation (Mutegi et al., 2008). The shading effect has the potential to establish a buffer for humidity and temperature, thereby safeguarding the system against severe weather conditions like heavy rainfall and heat waves (Schwendenmann et al., 2010).

1.3 HERBACEOUS STRIPS IN AGROFORESTRY

The herbaceous strips found in the agroforestry tree's understory are a significant area of undisturbed surface that maintains a permanent ground cover, responsible for the majority of roots (Cardinael et al., 2015a; Battie-Laclau et al., 2019) and increased soil organic carbon (Udawatta et al., 2017; Cardinael et al., 2015b) within the top centimeters of the soil beneath tree rows (Cardinael et al., 2015b; Battie-Laclau et al., 2019). These strips have the potential to significantly contribute to the preservation of arbuscular

mycorrhizal communities and overwintering of soil fauna in the tree row, which could subsequently migrate to the crop alley (Boinot et al., 2019). A greater abundance and biomass of earthworms in the tree row of a silvoarable system compared to the cropped alley was reported (Price & Gordon 1999) and linked to the influence of tree shading, reduced soil temperature, increased soil moisture, decreased soil disturbance, and greater availability of organic matter as a food source (Hauser et al., 1998; Araujo & López-Hernández, 1999; Solis et al., 2020). Studies have shown that the presence of trees in silvoarable systems generally leads to higher levels of soil organic carbon compared to agricultural fields without trees (Chatterjee et al., 2018; de Stefano & Jacobson, 2018; Feliciano et al., 2018; Shi et al., 2018; Cardinael et al., 2018), particularly along the tree rows (Bambrick et al., 2010; Wotherspoon et al., 2014; Cardinael et al., 2015).

Tree rows have the potential to serve as favorable habitats for the development of earthworm communities, particularly for epigeic and anecic earthworms, due to the lower level of soil disturbance, higher food availability, and buffered microclimatic conditions (Shipitalo et al., 1988). This could potentially facilitate the colonization of cropped alleys by earthworms. However, research on alley cropping systems that examine tree row effects frequently lacks distinct differentiation between the impacts of the trees and those of the understory herbaceous strip. Also, the documentation on the effects of trees and herbaceous strips and their

potential implications for crop nutrition and yields in agroforestry systems needs further attention.

1.4 NITROGEN LEACHING AND ITS PROBLEM

Nitrogen leaching, a prevalent concern in modern agriculture, takes center stage in my Phd. In this section, I examined the complex processes through which nitrates, especially through leaching, impact our ecosystems. This section serves as a fundamental cornerstone in my research journey, shedding light on the challenges posed by nitrogen leaching and, in turn, paving the way for an investigation into how agroforestry may offer sustainable solutions. I navigated through the relationships of nitrogen dynamics, land management, and ecological well-being, all with the ultimate goal of contributing valuable insights to the sustainable future of agriculture.

1.4.1 The nitrate leaching concept

Nitrate leaching refers to the process by which nitrate becomes dissolved and moves downward within the soil profile, facilitated by the percolation of water (Cameron et al., 2013; Gillette et al., 2018). Leaching could lead to the loss of nitrate that are leached beyond the vegetation's rooting zone (Ter Steege et al., 2001; Vitousek et al., 2009) resulting in their temporary removal from the system. However, there is a possibility of their recycling if roots extend deeper into the soil (Isaac and Borden, 2019).

The available nitrogen in the soil for plant use is typically in the form of mineral N, which can be either nitrate (NO_3^-) or ammonium (NH_4^+), with the ammonium being subsequently transformed into NO_3^- by soil microbes through the nitrification process (Cameroon et al., 2013). Over 50% of the mineral N supplied to crops can be lost to the environment (Cannavo et al., 2013). The potential loss of mineral N in soil is primarily attributed to three factors: (a) volatilization of ammonia (NH_3), which refers to the release of gaseous NH_3 from the soil surface; (b) denitrification and gaseous emissions of nitrogen, predominantly in the form of dinitrogen gas (N_2) and nitrous oxide (N_2O); and (c) leaching (Cameroon et al., 2013).

The predominant form of nitrogen loss through leaching is NO_3^- (Li et al., 2007; Di & Cameroon, 2003; Cao et al., 2014), although in sandy soils, there may be some leaching of NH_4^+ (Moreno et al., 1996). Nitrate, which is water-soluble, is susceptible to leaching due to the repulsion of the negatively charged NO_3^- anion by the negatively charged surfaces of clay minerals and soil organic matter (Arcelay, 2005). The retention and movement of nitrate in the soil solution within the soil are facilitated by the percolation of rainfall or irrigation (Gianquinto et al., 2013). The problem of nitrate leaching is frequently linked to the excessive application of chemical fertilizers in agricultural crop production (Cameron et al., 2013; Fowler et al., 2013; Lemaire & Gastal, 1997; Pratt, 1984; Padilla et al., 2018; Cui et al., 2020). Additionally, soil nitrate that is susceptible to leaching is also

generated by microorganisms that decompose plants and other nitrogen-containing residues present in the soil (Burgos et al., 2006; Biernat et al., 2020).

1.4.2 Impacts of nitrate leaching

Aside from the decline in soil fertility associated with nitrate leaching, it also poses a significant risk to both the environment and human health (Hester et al., 1996; Ju et al., 2006; Cameron et al., 2013; Watanabe et al., 2018). The introduction of nitrate into bodies of water such as rivers or lakes has the potential to contribute to the process of eutrophication, a phenomenon that leads to the proliferation of algae and a subsequent decline in fish populations (Boesch et al., 2001; Ngatia et al., 2019). Furthermore, nitrate leaching has been linked to serious health problems such as methemoglobinemia, also known as the 'blue baby syndrome,' in neonates, as well as gastric cancer in adults.

Agriculture in the EU contributes 40–80% of the total nitrogen entering surface waters (OECD, 2001), making it the region's largest single source of nitrate pollution. The annual cost to the EU of the environmental impacts of nitrogen pollution has been estimated to be around 70 to 320 billion euros (UWE 2013). Many efforts, including the nitrate directives and water framework directives that compel the EU member states to implement national action plans for monitoring, evaluation, and reduction of N losses,

have been implemented (European Commission, 2013)

1.4.3 Factors affecting nitrate leaching

Nitrate leaching is influenced not only by nitrogen inputs but also by the interactions between nitrogen processes and soil water balance (Pratt, 1984; Moreno et al., 1996; Ter Steege et al., 2001), which are influenced by the concentration of NO_3^- in the soil during the drainage period (Cameron et al., 2013; Ter Steege et al., 2001), as well as by the volume of water that passes through the soil (Pratt, 1984; Cameron et al., 2013). Apart from the soil NO_3^- concentration and drainage volume, several other factors, such as crop characteristics, soil type, and cropping methods, contribute to the potential for nitrate leaching (Ter Steege et al., 2001; Di and Cameron, 2002). The impact of soil properties on nitrate leaching is attributed to their influence on the hydrological processes governing water movement. Fine-textured soils generally exhibit lower nitrate leaching losses compared to coarse-textured soils due to their slower drainage and higher potential for denitrification (Di and Cameron, 2002; Borchard et al., 2019)

1.4.4 Management strategies aimed at mitigating nitrate leaching

Management strategies that could mitigate nitrate leaching include reduced nitrogen fertilizer rate, nitrification inhibition, partial organic substitution (livestock manure and crop residue) for synthetic nitrogen fertilizer, reduced water input rate, comprehensive management of water and fertilizer and use

of catch crops as well as agroforestry (Palma et al., 2007; Pavlidis & Tsihrintzis, 2018; Bai et al., 2020)

1.5 NUTRIENT ACQUISITION AND INTERACTIONS IN AGROFORESTRY

1.5.1 The nutrient safety net concept

The primary understanding behind the nutrient safety net is that the presence of trees in agroforestry can enhance nutrient retention in the soil-plant system and its conversion into biomass and harvested products, thereby preventing leaching (Young, 1997; Van Noordwijk & Lusiana, 1999; Rowe et al., 2001; Bergeron et al., 2011). The safety-net hypothesis posits that it is feasible to attain a decrease in nutrient leaching without intensifying root competition among trees and crops. Trees with minimal superficial roots and extensive deep roots that spread horizontally beneath the rooting zone of shallow-rooted crops form a theoretical safety net for leaching nutrients. In the subsoil, interception of nutrients and water occurs, resulting in a decrease in nutrient leaching. A study focused on examining the ability of *Peltophorum dasyrrhachis*, a deep-rooting and uncompetitive tree species, on utilizing subsoil nitrogen in conjunction with groundnut cultivation shows that the tree was able to acquire over 40% of its nitrogen from beneath the rooting zone of the crop (Rowe et al., 1999). Seyfried and Rao (1991) revealed that the soil solution in a multistate agroforestry system comprising cocoa, banana, and *Cordia alliodora* exhibited lower nutrient concentrations, resulting in reduced nutrient leaching as compared to a

maize monocrop in Costa Rica. Implementation of hedgerow intercropping with *Leucaena leucocephala* and annual food crops resulted in reduced nitrate concentrations in the soil solution and subsequently decreased leaching in southern Benin as compared to the agricultural control treatments (Horst, 1995). In a study conducted by Lehmann et al. (1999) in northern Kenya, it was observed that the intercropping of *Acacia saligna* and sorghum resulted in reduced nutrient leaching as compared to pure sorghum cultivation with runoff irrigation.

1.5.2 Vertical and horizontal acquisition of resources and root plasticity in agroforestry

The vertical distribution of plant roots tends to exhibit a greater concentration of roots in closer proximity to the soil surface, with a subsequent decrease in density as depth increases, which is consistent with the general distribution of soil nutrients (Jobbágy & Jackson, 2004). It has been observed that the architecture of root systems can exhibit significant variations among different species (Das & Chaturvedi, 2008; Borden et al., 2017). Rowe et al. (2001) revealed that in a tropical hedgerow agroforestry system with N isotope application, the uptake of ^{15}N decreased considerably with depth for the N_2 -fixing tree (*Gliricidia sepium*) with shallower roots. On the other hand, the deeper-rooted tree species (*Peltophorum dasyrrachis*) exhibited consistent uptake of ^{15}N from all depths up to 65 cm. Bouttier et al. (2014) observed a decrease in fine root length densities in the vicinity of

shallow-rooted poplar hybrids as opposed to intercropping with deeper-rooted oak trees (*Quercus rubra*). Hence, the principal approach for achieving optimal nutrient interactions in management involves the selection of tree species that possess complementary root distributions to those of crops, typically characterized by deeper-rooted trees.

The spatial arrangement of trees and crops plays a significant role in controlling nutrient acquisition in the horizontal dimension, such as root growth in tree rows versus crop rows (Thevathasan & Gordon, 1997; Jose & Gillespie, 1998; McGrath et al., 2001; Mora & Beer, 2013). The influence of species and ontogeny, i.e., tree size, on nutrient acquisition is manifested in the variability of the horizontal span of the tree-crop interface. Bambrick et al. (2010) reported that in temperate tree-based intercropping systems (TBI), there were noticeable fluctuations in soil organic carbon and soil nitrogen levels due to the deposition of tree litter inputs, which were observed up to a distance of approximately 10 meters from the tree row. According to Issah et al. (2015), the proportion of crop nitrogen derived from a leguminous shrub (*Caragana arborescens*) with a fixed nitrogen content decreased from 40% to 20% as the distance from the shrub row increased from 4 meters to 20 meters in shelterbelt systems. The significant nutrient contributions coincide with a densely competitive area. A study conducted by Thevathasan et al. (2012) revealed a reduction in certain crop yields within a 2-meter radius of the tree row in a TBI system. Livesley et al.

(2000) have reported the existence of variation in maize rooting densities on a comparable spatial gradient. The study has revealed that crop root length decreases while tree root length increases in closer proximity to trees, indicating more intense competition as the distance between the two decreases. The study conducted by Rowe et al. (2001) found no discernible spatial effect or alterations in tree fine root length densities in relation to nitrogen uptake with increasing distance from hedgerows consisting of *Peltophorum dasyrrachis* and *Gliricidia sepium*.

Physical trenching and pruning can be employed as a means of altering nutrient acquisition patterns in relation to both depth and horizontal distance from the tree (Jose & Gillespie, 1998; Jose et al., 2000; Dupraz et al., 2019).

1.5.3 Acquisition of nutrients from deep soil in agroforestry

Deep soil nutrient absorption is crucial where the risk of nutrient leaching is high. Recent research suggests that these processes are essential for improving geochemical cycling in agroforestry systems (Pierret et al., 2016) and that they are highly related to soil water dynamics in the soil profile (Wu et al., 2016).

According to Bordron et al. (2019), *Eucalyptus grandis* had a specific root uptake potential (nutrient uptake per length of the root) that was highest at a depth of 50 cm for NO_3^- (^{15}N) and 3 m for potassium (K^+) and calcium (Ca^{2+}) using Rb^+ and Sr^{2+} as analogs, respectively. Bergeron et al. (2011) show that

poplar roots play an active NO_3^- safety-net role in a TBI system to a depth of 1 m. Studies that go deeper than 3 m are few, although deep root research reveals active tree roots 10 m or deeper in other managed tree environments, such as eucalyptus plantations (Laclau et al. 2001, 2013). This indicates that there are strong depth patterns for nutrient acquisition. These depths for nutrient uptake are common in agroforestry systems; shade tree roots have been found to extend down to depths of up to 3 meters in cocoa and coffee (*Coffea arabica*) agroforestry systems, respectively (Abou Rajab et al., 2018). Deep tree roots may absorb nutrients, deposit them as litterfall in shallower soils, and make them available to crops (nutrient pumping effect). Robertson (1994) modeled the significance of deep N uptake and redistribution to crops in an *Acacia Senegal*-*Sorghum bicolor* system, Sanchez (1995) lists deep nutrient capture as a key pathway for new N, and Hartemink et al. (1996) measure NO_3^- uptake from deep subsoils.

1.5.4 Atmospheric nitrogen fixation and N transfer in agroforestry

The development of nodules in the roots of leguminous trees through soil bacteria and the resulting process of biological nitrogen fixation have been found to significantly enhance the availability of nitrogen sources for crop uptake in agroforestry (Nygren et al., 2012; Munroe & Isaac, 2014; Kaba et al., 2019). The annual addition of nitrogen to an agroforestry system through N_2 fixation can range from tens to hundreds of kilograms per hectare and is influenced by various factors, including climate, soil

characteristics, mycorrhizal status, and management practices such as nutrient inputs, pruning frequency/intensity, and tree physiology (Beer et al., 1998; Nygren et al., 2000; Isaac et al., 2011).

The delivery of fixed nitrogen to crops in agroforestry occurs through two distinct pathways. The first is indirect and involves the decomposition and mineralization of organic compounds such as leaf litter, dead roots, and nodules. The second pathway is direct and involves the transfer of fixed N via root exudates, root transfer, and common mycorrhizal networks (Nygren et al., 2013). Kurppa et al. (2010) employed soil isotopic enrichment as a means to examine the transmission of nitrogen from leguminous shade trees, namely *Gliricidia sepium* and *Inga edulis*, to cocoa saplings in a semi-controlled field setting. The results indicated that all fixed-N transfer was attributed to belowground transmission. According to Issah et al. (2015), 40% of the yearly crop nitrogen uptake in *Caragana arborescens* shelterbelt systems was obtained from fixed nitrogen, as determined by means of ¹⁵N isotopic dilution. Root-root interactions in a leguminous tree and grass systems have demonstrated varying rates of nitrogen transfer. The study conducted by Rao and Giller (1993) demonstrated a contribution of approximately 3% between *Leucaena diversifolia* and *Cenchrus ciliaris*. Similarly, Jalonen et al. (2009) conducted a greenhouse study that revealed that *Dichantium aristatum* absorbed 22% of nitrogen exuded from the roots of *Gliricidia sepium*.

1.6 USE OF TRACERS AND MODELS FOR ASSESSMENT OF NITRATE LEACHING IN AGROFORESTRY

1.6.1 The tracer method

Quantifying nutrient leaching using tracers can be conducted either by

1. Quantifying the tracer content in the soil up to a specific depth, then comparing the retrieved quantity with the initially applied amount (or the amount measured in the same soil volume at a prior date), and interpreting the disparity as leached or
2. Quantifying the tracer movement through a specific soil layer, such as the crop's maximum rooting depth.

There exists a considerable variety of distinct tracers, which may be categorized as follows:

- Radioactive isotopes such as ^{32}P , ^{33}P and ^{35}S (Comar, 1955; Diwan et al., 2018)
- Stable isotopes (e.g. ^{15}N , ^{34}S) (Reynolds-Vargas et al., 2006 ; Hastings et al., 2013 ; Colombani et al., 2019 ; Ju et al., 2023)

The utilization of radioisotopes and stable isotopes enables the assessment of nutrient leaching in soil without causing significant changes in the concentration of the nutrient under investigation. Non-radioactive tracers have the ability to mimic the actions of specific chemically related nutrients in the soil. For instance, nitrate can be represented by chloride and bromide,

calcium by strontium, and potassium by rubidium and lithium (Gockele, 2014; Mamolos et al., 1995; Aebersold, 1953). Stable isotopes of nitrogen are often employed for the assessment of nitrate leaching (Dawson et al., 2002; Chalk et al., 2019; Du et al., 2022). This is because these isotopes exhibit comparable characteristics during decomposition and microbial transformation to the nutrient under investigation. In several instances, radioisotopes may be deemed excessively hazardous (Comar, 1955; Cipriani et al., 2020) for conducting an agricultural field experiment, thereby rendering stable isotopes as the sole viable alternative.

When the focus of the study is to evaluate the transportation of nutrients in the soil, as opposed to their release from a specific nutrient source, it is recommended that the tracer be administered in a solution form (Du et al., 2022). This is due to the fact that solution-based tracers can be distributed more uniformly compared to solid substances such as fertilizers. A defined area can be sprayed with a known quantity of dissolved tracer using either a basic hand sprayer or an electric pump. The quantity of application is dependent upon the initial soil concentration and the experimental timeframe. In order to investigate nutrient leaching subsequent to fertilization, substantial quantities of nutrients to the soil are applied, but there is the possibility of pool substitution of the tracer of the native soil nutrients (Jenkinson et al., 1985). The retrieval of tracers for analysis can be accomplished through various methods such as soil solution collection, soil

extraction using a salt solution or dilute acid, or direct analysis through dry combustion if nitrogen is the main target.

1.6.2 Use of Models

Numerical models have been used to simulate nitrate leaching (extensively reviewed in Cui et al., 2020), nitrogen fertilizer application as well as water and nitrogen balance in a cropping system (see table 2). Nitrate leaching from fertilizer applications has been predicted using the Hydrus-2D/3D model (Baram et al., 2016). The model was applied to simulate future nitrate changes in Israel's coastal regions. In the model, nitrate concentration in the groundwater remained constant for 40 years after the fertilizer rate was lowered by 25%. However, the groundwater's nitrate concentration was lowered to safe level when the quantity of fertilizer was decreased by 50% (Kurtzman et al., 2013; Levy et al., 2017). Fertilizing in accordance with crop needs is crucial for decreasing nitrate leaching (Riga & Charpentier, 1999). Pinto et al. (2017) simulated the link between N fertilizer input and nitrate leaching using the ANIMO model (Wolf et al., 2005). According to the modelling findings, nitrate leaching decreases by 28% to 47% when the N application rate is lowered from 600 to 400 kg N ha⁻¹ yr⁻¹. In order to show that nitrate leaching will be greatly decreased when the input of N in apple orchards is less than 100 kg N ha⁻¹, Demestihias et al. (2018) also employed the STICS model.

According to another model called Hydrus-2D/3D model, switching from sprinkler irrigation to drip irrigation will minimize nitrate leaching by over 37% (Hardie, 2017; Phogat et al., 2014). The root zone water quality model (RZWQM2), like the Hydrus-2D/3D model, is excellent at simulating how irrigation plans would affect nitrate leaching (Craft et al., 2018). The transport of water and solutes in unsaturated or partially saturated soils is simulated using the Leaching Estimation and Chemistry Model (LEACHM) (Asada et al., 2018). It is a useful tool that helps in studying how nitrogen is transported and converted in soils. To simulate the N dynamics in Mediterranean citrus orchards, Lidon et al. (2013) simulated using the LEACHM model, which predicted nitrate leaching with high level of accuracy. In order to simulate nitrate content in soil, Contreras et al. (2009) utilized a one-layer and a three-layer model (a simplified model with few parameters), and they compared the outcomes with those of the LEACHM model. The findings demonstrated that the three models' simulations of soil nitrate concentration were close to the actual amount. The three-layer model, however, provides higher accuracy and better prediction effects than the one-layer model.

Models have limitations. For instance, weeded tree rows and grasslands in alleys cannot compete for water and nitrogen in the STICS model (Demestihias et al., 2018; Yin et al., 2020). The contribution of seasonal storage and removal of nitrogen or soil mineralization to available nitrogen

in the Hydrus 2D/3D model cannot be explained by the tree structure (Shafeeq et al., 2020). According to Cameira et al. (2014) and Zhang et al. (2017), RZWQM2 and HEQM models respectively includes a lot of parameters and is challenging to calibrate. In many annual crops, the LEACHM model is frequently utilized. However, it is not often in perennial crops (Lidon et al., 2013). The research goals, the model's usability, and a fair balance between fewer parameter needs and robustness should all be considered when selecting a model.

Table 2: A list of some nitrate leaching models and their references

Model name	Reference
Nmin Sytem	Ramos et al., 2002
HYDRUS-2D	Levy et al., 2017.
HYDRUS-1D	Phogat et al., 2014.
STICKS	Demestihias et al., 2018.
RZWQM2	Nolan et al., 2010
Episties	Nesme et al., 2006; Nesme et al., 2009
HI	Dzurella et al., 2015.
WAVE	Riga and Chapentier, 1999.
LEACHM	Lidon et al., 2013.
HEQM	Zhang et al., 2017.
Hi-sAFe	Dupraz et al., 2009

1.7 AGROFORESTRY UNDER CLIMATE CHANGE

In a world where climate change is an undeniable reality, the relationship between agroforestry and the evolving climate landscape is of paramount importance. This section is a pivotal stepping stone, marking the intersection of my primary research focus on nitrogen fluxes and the broader context of agroforestry's resilience in the face of climate change. Agroforestry holds the potential to not only mitigate nitrate leaching but also adapt to and mitigate the impacts of climate change, thereby contributing to a more sustainable and climate-resilient future for agriculture.

Climate change is anticipated to have a significant impact on crop yields, especially in Mediterranean regions, where yield reductions of up to 70% are expected primarily due to rising temperatures (Olesen & Bindi, 2002; Maracchi et al., 2005; Lobell et al., 2012; Asseng et al., 2015; Mistry et al., 2017). However, when it comes to agroforestry systems, the outlook appears different. Future climate scenarios suggest that agroforestry systems will experience reduced stress factors such as heat, drought, and nitrogen stress, leading to more stable crop yields (Gomes et al., 2020; Reyes et al. 2021).

While the negative effect of climate change on agroforestry crops may be lower compared to monoculture, questions arise regarding the impact on trees themselves. Trees play a crucial role in global climate regulation, and their response to increasing atmospheric CO₂ levels is key to mitigating

climate change (Read et al., 2009). Elevated CO₂ levels have been found to enhance tree photosynthesis by improving the carboxylation rate of Rubisco and concurrently suppressing the oxygenation of Ribulose-1,5-biphosphate (RubP) (Drake et al., 1997). Exposure to elevated CO₂ concentrations can result in a 31% increase in photosynthesis rates (Ainsworth & Long, 2004). Additionally, elevated CO₂ has been shown to enhance the intrinsic water use efficiency in trees (Ainsworth & Long, 2005).

However, understanding how trees respond to environmental factors, especially rising CO₂ levels, becomes complex when incorporated into the context of agroforestry. This complexity arises from the interactions underlying the mechanisms governing processes like photosynthesis, tree growth, and water use. While the response of plants to individual factors like CO₂, water, and temperature is well-established, comprehending the intricate interactions and their implications for trees' contribution to environmental regulation such as nitrogen cycle, both now and in the future, is an ongoing area of research.

1.8 QUESTIONS TO BE ADDRESSED

As the world grapples with the urgent need for sustainable agricultural practices that can ensure food security, conserve natural resources, and mitigate the impacts of climate change, agroforestry has emerged as a promising solution. This PhD dissertation embarks on a comprehensive

exploration of the complex interactions within agroforestry systems, focusing on black locust and hybrid walnut trees species in Mediterranean environments. The key questions at the heart of this research revolve around the dynamics of nutrient management, the role of soil microbes, nitrogen cycling, and the incorporation of CO₂ effects into an soil-crop-tree model (Hi-sAFe). With climate adaptation and sustainable agriculture at the forefront, this study aspires to address these critical inquiries and contribute to the broader understanding of agroforestry's potential for a more resilient and ecologically sound future.

The research strategy adopted in this study is multi-faceted and interdisciplinary. It combines extensive field experiments, isotope labeling techniques, soil microbial analysis and modeling. These diverse methodologies provide a holistic view of the agroforestry system, enabling the investigation of nutrient safety nets, microbial contributions, nitrogen stock assesment, and the impact of elevated CO₂. By focusing on the integration of CO₂ effects into the Hi-SAFE model, this research advances the state of knowledge in agroforestry modeling. The collaborative efforts of experts from various fields, such as ecology, agronomy, soil science, and microbiology, underline the importance of interdisciplinary approaches in addressing the complexities of sustainable agriculture and climate adaptation.

This PhD manuscript is built into chapters 1 to 5 as follows:

In Chapter 1, I provided an introduction to the field of study and present a comprehensive literature review. This chapter aims to establish the context for the research, highlight key concepts, and identify research gaps that serve as the foundation for the subsequent chapters.

Chapter 2 which is titled “Assessing Nutrient Safety Net and Crop Yield in a Mediterranean Agroforestry System” discusses the first-year research, outlining the objectives and hypotheses, the methodology employed for the ^{15}N labeling experiment, presented the results obtained, engaged in a discussion of these findings, and concluded with the implications for agroforestry systems.

Chapter 3 builds on the second year of the study, conducting a 2-year assessment of tree nutrient safety nets, nitrogen stocks, and biological nitrogen fixation (NDFFA) in a Mediterranean agroforestry system. I discussed the extended ^{15}N labeling experiment, presented the results, engaged in a thorough discussion of the findings, and conclude by outlining the implications for nutrient management in agroforestry.

The focus of Chapter 4 was the third-year research, which explores the response of hybrid walnut trees to elevated CO_2 in agroforestry using Hi-SAFE model. I detailed the objectives of the study, describe the model and its integration with CO_2 effects, and presented the preliminary research

findings and observations. The chapter concludes by offering future directions and recommendations for agroforestry in a changing CO₂ environment.

Chapter 5 serves as the culmination of the manuscript. It includes a summary of key findings from all research chapters, a comparison of these findings to existing literature, methodological reflections that highlight the strengths and weaknesses of the research methodologies, identification of unanswered questions, insights into the broader significance of the research in the context of science, society, and the world, and an overall conclusion that underscores the contributions to the field of agroforestry.

In the references section at the end of the manuscript, citations for sources used in Chapter 1, Chapter 2, Chapter 3, and Chapter 4 were provided to give credit to prior research and sources that have informed this work.

2 CHAPTER 2: ASSESSING NUTRIENT SAFETY NET AND CROP YIELD IN A MEDITERRANEAN AGROFORESTRY USING ¹⁵N LABELLING EXPERIMENT

Paper submitted to Soil Science and Plant Nutrition on 9/10/2023

Title: Assessing nutrient safety net and crop yield in a Mediterranean agroforestry using ¹⁵N labelling experiment

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Abstract

Purpose: This study aimed to assess crop yield and soil nitrogen removal by trees from a potential nitrogen leaching pool in Mediterranean agroforestry, in a ¹⁵N-labeled fertilizer field experiment. We hypothesized that the applied ¹⁵N would be taken up by the trees, understory vegetation strip (UVS), and soil microbial biomass, and that crop yield would be reduced in agroforestry

systems.

Methods: In a two-block agroforestry design, ^{15}N -labeled urea was applied to the crop-soil surface in agroforestry and monocrop plots. We analyzed the ^{15}N , nitrogen, and carbon contents of the aboveground and belowground compartments of the trees, crops, and UVS, as well as that of the soil microbial biomass.

Results: No labelled ^{15}N was found in the trees, UVS and microbial biomass. Additionally, agroforestry-crop yielded a -20% lower yield compared to monocrop. The absence of ^{15}N uptake by trees was attributed to the tree age and rooting pattern, which resulted in the lack of tree roots in contact with the labelled fertilizer. The absence of labelled ^{15}N in the microbial biomass indicated a preference for pre-existing organic matter over newly generated ^{15}N from urea mineralization.

Conclusions: The capacity of agroforestry systems on crop yield and potentially leachable nitrate may be influenced by the age of trees and prevailing environmental and management practices. Further investigations after several years of tree growth are necessary to better understand the long-term effects of tree age on crop yield and the nutrient retention capacity, including the response pattern of nitrogen-fixing trees.

Keywords Agroforestry, *Robinia pseudoacacia*, microbial biomass, biomass partitioning, nitrogen allocation, ¹⁵N labeling,

Abbreviations and definition

DBH: Diameter at Breast Height

DOY: Day of the Year

UVS: Understory Vegetation Strip

1 Introduction

Due to its capacity to boost crop yields, nitrogen is a crucial nutrient for agriculture. That has led to the global consumption of nitrogen fertilizer growing steadily since the 20th century (FAO, 2019; Foley et al., 2011). However, more than half of the N input to agricultural fields is lost into the environment via several pathways, including leaching, runoff, and greenhouse gas emissions (Velthof et al., 2009; Autret et al., 2019; Omara et al., 2019). Agriculture in the EU contributes 40-80% of the total nitrogen entering surface waters (OECD, 2001), making it the region's largest single source of nitrate pollution. It is causing environmental degradation (surface and groundwater pollution) and human health impacts (Zhai et al., 2017; Ward et al., 2018; Zhang et al., 2021).

Regarding agricultural nitrogen leaching, agroforestry has been identified as one of the best mitigation options (Reisner et al., 2007; Lawson et al., 2020). Agroforestry is defined as a land-use system where trees are grown in combination with crops or grasslands on the same land (Somarriba, 1992; European Commission, 2013). Trees in agroforestry could affect nitrogen dynamics by changing the soil's physical, chemical, and biological properties and play a role of a safety-net by capturing the nitrogen that would otherwise be lost by leaching (Lawson et al., 2020; Sileshi et al., 2020). Indeed, trees rows may intercept a much higher amount of nitrogen

thanks to their deeper roots than that of crops (Jose 2009; Andrianarisoa et al. 2016) and leading to a lower nitrogen flux toward subsequent groundwater (Jose 2009; Pavlidis and Tsihrintzis 2018). However, it is important to recognize that the complete understanding of the safety-net role of trees in nitrogen interception is nuanced. Several factors come into play, including the age of the trees, their specific genotype, and the prevailing pedoclimatic conditions (Schroth, 1998; Van Noordwijk et al., 2015). These factors can influence the capacity of trees to capture leachable nitrogen effectively, understanding them is essential for optimizing the benefits of agroforestry systems and sustainable land management practices.

In alley cropping agroforestry systems, three types of plant groups are associated: the trees, the understory plant species beneath them, and the arable crop under cultivation (Guillot et al., 2021; D'Hervilly et al., 2022; Siegwart et al., 2022). A risk associated with agroforestry is a decrease in crop yield due to the competition for light, water and nutrients that trees and their understory vegetation can impose on the nearby crop. These plant communities are heterogeneous in terms of capture and utilization of soil nutrients. The arable crops mostly take up nutrients from the top soil (Fan et al., 2016; Chen et al., 2019; Sun et al., 2022), while the trees and herbaceous strips, which are predominantly perennials, tend to have deeper soil exploration capabilities and higher root biomass than the annual crops (Beniston et al., 2014; Ferchaud et al., 2015; Duchene et al., 2020). The

effective spatial distribution (overlapping or independence) of root systems will direct the relationships between different groups of plants towards complementarity or competition for water and nutrients (Van Noordwijk, et al., 2015).

Agroforestry enhances the development of soil microbial biomass via the presence of tree roots and its understory vegetation, which could improve soil nitrogen retention (Araujo et al., 2012; Beule et al., 2022). Indeed, root development allows the growth of microbial biomass in the rhizosphere (Bloor et al., 2009; Battie-Laclau et al., 2020), which constitutes a specific microbiota and microbial C and N reservoir. Immobilization of nitrogen by the rhizospheric microbial biomass is expected under the influence of carbon inputs linked to root rhizodeposits (Diaz et al., 1993; Hungate, 1999). The nitrogen contained in the microbial biomass then constitutes a temporary nitrogen reservoir that can be returned to the soil during microbial turnover. The microbial biomass of the soil can represent around 100 kg N ha⁻¹ (Rennenberg and Dannenmann, 2015; Maxwell et al., 2020). Soil microbial biomass could therefore serve as an important nitrogen sink which can contribute to retention of soil leachable nitrate in the upper horizon of the soil.

In addition to increased soil microbial biomass, nitrogen-fixing trees such as *Robinia pseudoacacia* were shown to improve soil fertility via the return of

symbiotically fixed nitrogen to the soil through litter fall, N-enriched root and nodule decomposition (Ntayombya and Gordon, 2015; Marron et al., 2018). This improvement may potentially reduce the reliance on organic or mineral nitrogen fertilization of intercropped plants in the long term (Dommergues, 1987; Araujo et al., 2012). Moreover, nitrogen-fixing trees might be less dependent to soil N for their N supply, and thus they might be less effective nutrient safety nets than non-legume trees, in particular for nitrates.

To ascertain the true benefits of agroforestry, it is crucial to quantify nitrogen fluxes and compare yields obtained in agroforestry systems with those achieved in monocultures. The assessment of nitrogen fluxes using ^{15}N tracers is a highly effective methodology for testing the nitrogen safety net hypothesis on agroforestry trees and evaluating the applied nitrogen fertilizer utilization by crops, grasses, and soil microbes in agroforestry systems (Cannavo et al., 2013; Rowe et al., 2005). This approach is particularly valuable because soil N mineralization leads to the formation of additional inorganic nitrogen, making it challenging to measure directly the fate of applied mineral fertilizer. However, the presence of multiple biomass compartments in the system necessitates the assessment of nitrogen partitioning within each component. Perhaps due to these complexities, only a limited number of studies have utilized this technique to investigate

nitrogen fluxes in agroforestry systems (Nannipieri et al., 1999; Lehmann et al., 2004; Rowe et al., 2005; Cannavo et al., 2013).

To the best of our knowledge, there is a lack of field studies examining nitrogen stocks and fluxes in young agroforestry sites. The aim of this study was to investigate fertilizer derived-N acquisition by arable crops, nitrogen-fixing trees, understory vegetation and microbial biomass in a young agroforestry system compared to monoculture under a Mediterranean climate, using a ^{15}N -labeled fertilizer. We formulated three hypotheses: Firstly, we hypothesized that UVS and tree root systems explore toward the cropping alley, allowing both compartments to absorb ^{15}N applied in the crops. Secondly, in agroforestry systems, the coexistence of trees alongside crops may lead to lower crop yield compared to monocrop systems, due to competition for resources such as water, light, and nutrients which could lead to increased leaching. Thirdly, we hypothesized that microbial biomass would act as a nitrogen sink, especially within agroforestry systems, attributed to the increased root density resulting from the combined presence of crops, understory vegetation, and trees.

2 Material and methods

2.1 Study area: soil and climate characteristics, cultural practices

The study was conducted at an INRAE Instrumented Agroforestry Site (Dispositif Instrumenté en Agroforesterie Méditerranéenne Sous contrainte hydrique, DIAMS) located 10 km East of Montpellier, France. Over the period 2012–2021, the maximum, mean, and minimum temperatures of the site were 33.1°C, 15.5°C, and -3.6°C, respectively, and the mean precipitation was 556 mm (Mauguio 2012–2021, INRAE Station data). The cumulative rainfall between the periods of labeling and sampling (April 29 to June 21, 2021) was 53.5 mm (Figure 1). The soil was classified as a skeletal rhodic luvisol (WRB) with a high proportion of stones (up to 60%) (see Siegwart et al., 2022 for further description, Table A). For the purpose of this study, the soil layer was limited to 0–20 cm. The pH_{water} range at 0–20 cm depth was 6.8–7.1. The study area (total area of 5 hectares) comprised one plot of alley agroforestry (AF) and one plot of monocrop (MC), repeated in three independent blocks, considered as independent replicate. For our study, only two blocks were used (Figure 2 A).

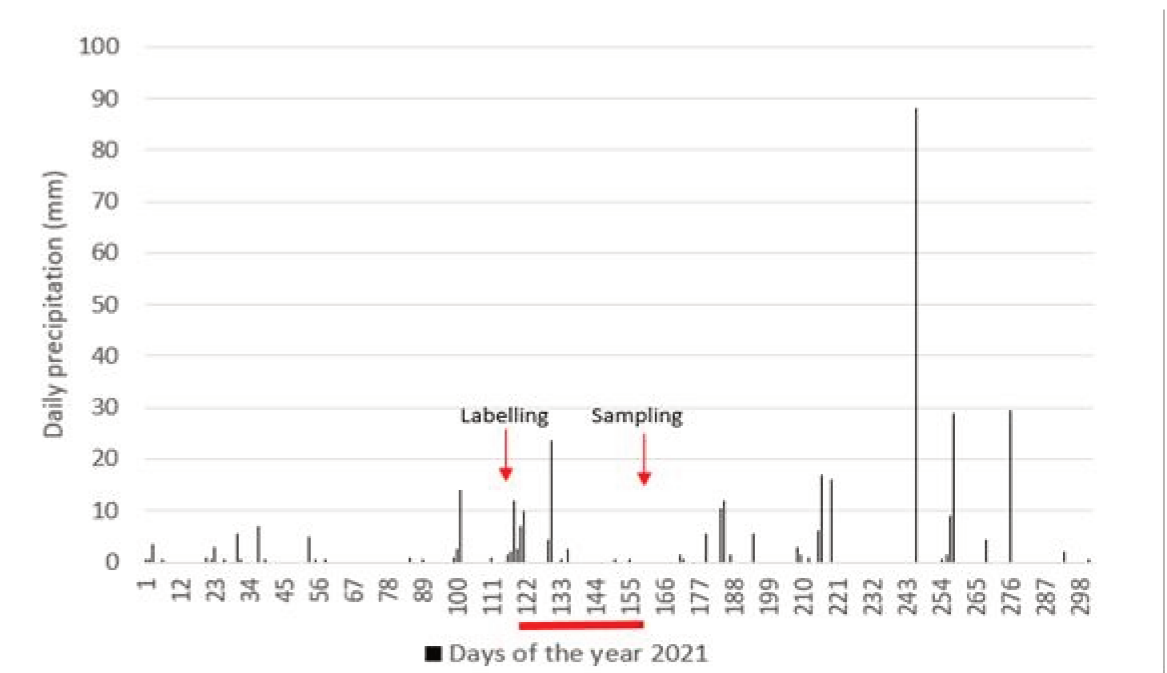


Fig. 1: 2021 precipitation distribution at the DIAMs site, Montpellier.

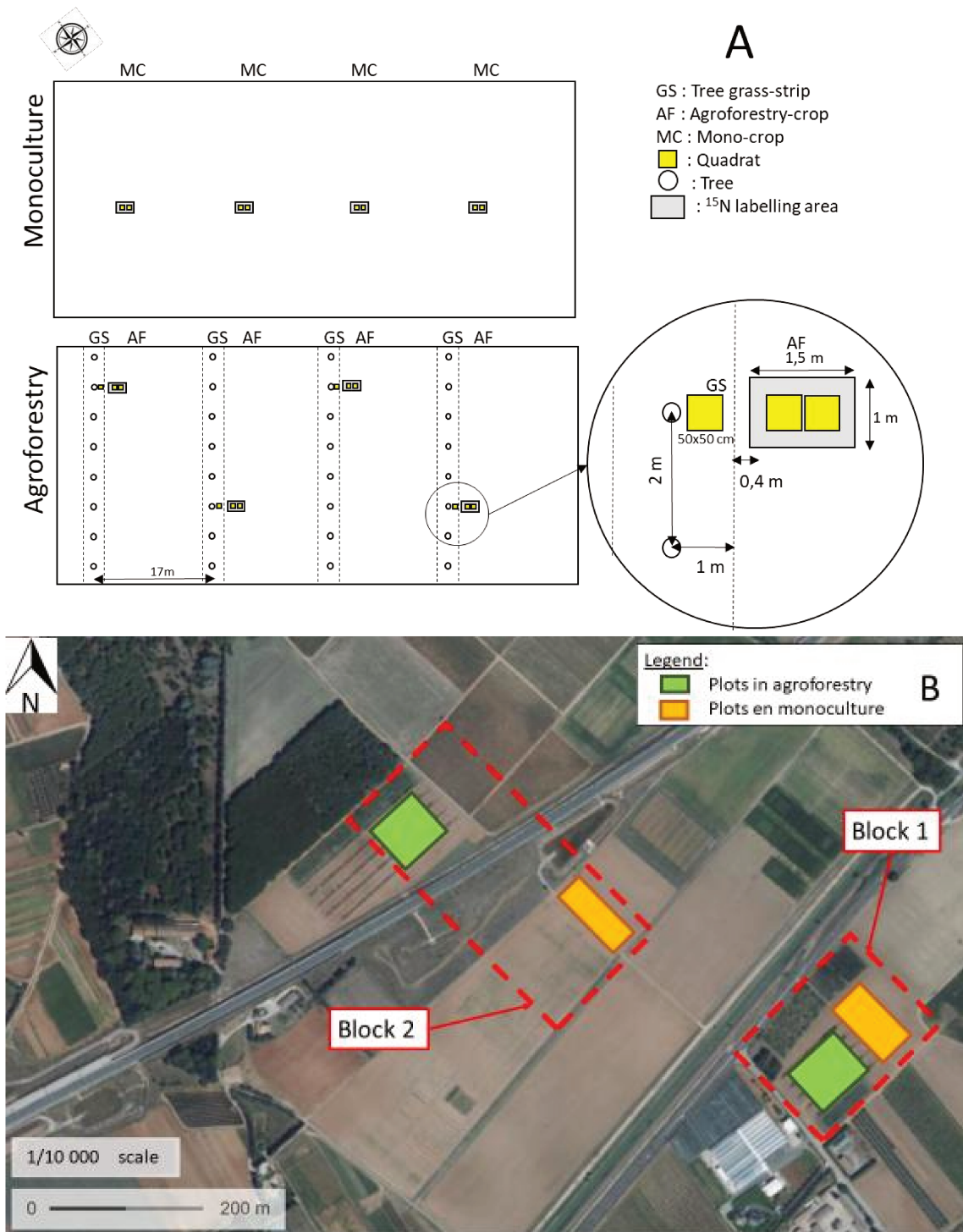


Fig. 2: Aerial view of DIAMs site in Maugio showing two blocks containing agroforestry and monocrop plots (A) and experimental design of

block 1 (B).

The alley-cropping system was planted in 2017 with Black Locust (*Robinia pseudoacacia* L.), a N-fixing species, in 2-m-wide rows covered with an understory vegetation strip (UVS). The trees were spaced (intra-row) by 2 meters, and rows were 17 m apart (each row containing 44 trees), leading to a density of 294 trees ha⁻¹. The mean tree DBH in 2021 was 4.08 ± 1.2 cm, while the mean height was 3.18 ± 0.85 m (for block 1 and 2). Herbaceous species *Festuca arundinacea*, *Dactylis glomerata*, *Medicago sativa* and *Trifolium pratense* were sowed in 2019 to create the UVS, delimiting the tree row. However, other herbaceous species such as *Bromus sp.*, *Papaver rhoeas*, *Crepis sancta*, *Sonchus arvensis*, and *Lolium perenne* were also identified on the UVS by Siegwart et al., (2022). There was a 0.4 m gap of bare soil between the limits of the UVS and the crop alley.

In both AF and MC, barley (*Hordeum vulgare*) was sown on January 22nd (block 1) and 21st (block 2) of 2021. A first dose of Smart N 46 fertilizer was applied at a rate of 87 kg ha⁻¹ (equivalent to 40 kg N ha⁻¹) on the 4th of March followed by a second dose of 60 kg N ha⁻¹ in April making the total nitrogen quantity added to be 100 kg N ha⁻¹. Crop harvest was on the 30th of June (block 1) and the 2nd of July (block 2).

2.2 Experimental design

In each modality of cultivation (AF and MC) of each block, four experimental areas were chosen as four replicates (Figure 2B).

In the AF sites, one tree per row (and four rows were chosen) was selected which served as the reference point for the tree, crop, soil, and UVS sampling (Figure 2B). Trees were chosen according to similar diameter and height (i.e. 4.06 ± 0.17 cm, 3.40 ± 0.44 m, respectively). In the MC sites, the four experimental areas were chosen so as to be distributed along the plot and separated by 17 m (as in agroforestry). In each experimental area, a ^{15}N labelling of the crop soil was performed and the ^{15}N was further followed in the different compartments of the tree, UVS and crop.

2.3 ^{15}N labeling

^{15}N labeling was carried out on April 29, 2021, Day of the Year (DOY) 119, which coincided with the second dose of fertilizer application (done on April 26). A quadrat with a dimension of 1 m x 1.5 m was used to delimit the ^{15}N labeling area (Figure 2). That dimension was chosen in order to minimize the border effect during crop sampling (see below). In the agroforestry plots, the labeled areas were placed perpendicular to the tree trunk at a distance of 1.4 m. In the monocrop, four ^{15}N labeling areas per block were chosen, spaced 17 m apart (Figure 2). The location of each labelling area was identified by its GPS coordinates to avoid sampling

unlabeled points. These locations were used for both initial and subsequent sampling.

A ^{15}N -labeling solution was prepared with 24 g of urea ($\text{CH}_4^{15}\text{N}_2\text{O}$) containing 98% ^{15}N in 1.7 l of water. For each ^{15}N labeling area, 100 ml of that solution was put in 4.9 l of water. These 5 liters were applied in each ^{15}N labeling area (by homogenous spray on the soil using a manual pressure sprayer) and thereby added 0.4607 moles of ^{15}N per plot, equivalent to 0.46 g of ^{15}N per m^2 , i.e., 4.6 kg of ^{15}N ha^{-1} .

2.4 Plant and soil sampling

Two sampling campaigns were carried out for trees (leaves, branches, and roots), soil and the shoot and root parts of understory vegetation and crops. The first sampling was conducted a few days prior to labeling in April (26th–28th of April 2021, 95–97 days after sowing). During the same period, crop density in AF and MC were measured with a quadrat of 0.5 m x 0.5 m dimensions. The number of stalks per seed was also recorded on 10 seeds per replicate. The second sampling campaign was carried out one day before the barley harvest (the 29th of June, 2021). In addition to the crop's leaves and roots, barley spikes were also sampled in the second harvest.

For each tree, 3-5 leaf-bearing branches well distributed in the tree canopy were cut with pruning shears, after which the leaves and the branches were

separated and put inside separate bags. For the tree root sampling, the soil at the trunk base was gently dug up to 20 cm deep with a digger and shovel to expose the tree fine and medium roots. Black locust roots were easily identified by their distinct color and smell. The vegetative samples were dried and ground, pending total N and ^{15}N determination.

For crop sampling, two small quadrats each of 0.5m x 0.5m, were put inside the ^{15}N labeling area, and the barley plants inside them were cut with shears and oven-dried. The respective weights of the tiller and spike in the quadrats were measured. The number of spikes per quadrat was counted. For crop root sampling, ten barley plants within the small quadrats were uprooted down to 20 cm depth, and the root systems were cut, washed and dried in an oven. Crop root density in the first 15 cm of depth expressed in g by m^2 was estimated by extrapolating the amount of roots quantified in a manual soil auger (15 cm x 8 cm) in each of the 4 replicates.

For UVS, the grass leaves and roots were sampled using a quadrat of 0.5m x 0.5m placed near the tree trunk, directly opposite the ^{15}N labeling area. The counter-faces of the ^{15}N labelling area and the quadrat were separated by 0.5 meters (Figure 2). The roots were cut with shears to separate them from the leaves. All samples were oven-dried at 65°C.

For soil, the first sampling was conducted on the UVS and 1 m away from the future labeled areas of the crop to assess the initial soil microbial carbon,

nitrogen, and ^{15}N statuses. The second soil sampling was conducted 63 days after labeling (on June 29, 2021) at the UVS and the labeled areas of the crop. Twelve soil samples were taken from each block using the manual soil auger (15 cm x 8 cm): four samples were taken near the tree trunk (at the center of each UVS sampling quadrat), four from the agroforestry crop and four from the monocrop (at the center of each labeling area). Soil was sieved to separate the soil into three categories: stones, soil particles greater than 1 mm, and fine soil less than 1mm.

Soil water content was determined by drying 5 g of fresh soil from each sample for 24 hours at 105 °C.

2.5 Microbial biomass assessment

Soil microbial N and C were measured using the chloroform fumigation extraction method (Brookes et al. 1985; Vance et al., 1987). Two fresh soil subsamples of 5 g sieved at 1 mm were prepared. One subsample was fumigated for 24 hours with chloroform vapor, while the other was not fumigated. Nitrogen and carbon extraction of both subsamples were performed using 20 ml of 0.5 mol l⁻¹ K₂SO₄ for 30 min under vigorous shaking at 250 rpm for N determination. For ^{15}N determination, the same procedure was used, but with extraction by 20 ml of 0.03 mol l⁻¹ K₂SO₄ to avoid interference of the mass spectrometer with the K₂SO₄ salt during analysis (Fontaine et al., 2004; Barnard et al. 2006). The extracts (fumigated

and non-fumigated) were filtered (GFC WHATMANN 1.2 μm mesh), then analyzed for N and C content using an elemental analyzer (TOC TNM-1, Shimadzu, Champs-sur-Marne, France) or freeze-dried for subsequent ^{15}N determination using an isotopic ratio mass spectrometer (FLASH 2000 HT/IRMS, Thermo Scientific).

The microbial biomass C was estimated as $[(\text{C in fumigated soil}) - (\text{C in non-fumigated soil})] / 0.45$ (Vance et al., 1987). The microbial biomass N was estimated as $[(\text{N in fumigated soil}) - (\text{N in non-fumigated soil})] / 0.54$ (Brookes et al. 1985). The ^{15}N concentrations were similarly determined from the fumigated and non-fumigated 0.03 mol l^{-1} soil extracts. In fumigated and non-fumigated samples, the excess ^{15}N (%) was determined as the difference between the ^{15}N % value of the sample and the % ^{15}N of the natural abundance of the sample before labelling. Microbial biomass originally expressed in $\mu\text{g C or N / g of dry soil}$ was converted into g C or N / m^2 using the volume of the corer (753 cm^3) and the bulk density of the soil (i.e. 0.47 g/cm^3 for fine soil).

2.6 Plant biomass sample preparation for N and ^{15}N determination

The tree branches, leaves, and roots; crop leaves, root, and spike; and UVS leaves and roots were ground to a fine homogenous powder ($80\mu\text{m}$) using Mixer Mill MM301, Retsch, for 1-6 minutes in 2 mL microtubes (Sarstedt) with two aluminum balls. The powder was then transferred into tin capsules

(D1006, Elemental Microanalysis) using a microgram balance (LE26P, Sartorius) (2 mg for leaves; 3 mg for branches; 4 mg for roots and spike). These samples were then analyzed for their total N concentration by dry combustion with an N analyzer (TNM-1, Shimadzu, Champs-sur-Marne, France) and their ^{15}N concentration with an isotopic mass spectrometer (FLASH 2000 HT/IRMS, Thermo Scientific, INRAE, Versailles).

2.7 Calculations

Quantity of N and recovered ^{15}N in the different compartments

The amount of nitrogen present in each compartment, expressed as N stock in g N was obtained by multiplying the concentration data (g N g⁻¹ of compartment) by the compartment's biomass.

The quantity of ^{15}N from the labelled solution that was recovered in the different compartments was calculated using the following formula:

$$\text{Recovered } ^{15}\text{N} = \text{Atom\% } ^{15}\text{N excess compartment.} * \text{N\% compartment} * \text{total biomass of compartment}$$

With $\text{Atom\% } ^{15}\text{N excess compartment.} =$

$$\text{Atom\% } ^{15}\text{N compartment after labelling} - \text{Atom\% } ^{15}\text{N compartment natural abundance}$$

We assumed that there were two different sources of N in the agroforestry site: the atmosphere (N fixation and N deposition) and the soil (addition

from inorganic and organic N sources such as fertilization, litterfall, and mineralization). Therefore, ^{15}N in excess was computed as the difference between the natural abundance of ^{15}N in the compartments measured before labeling and that after labeling, except for the spike, in which the cited natural abundance of 0.36 was used (Deléens et al. 1997) as the initial.

2.8 Statistical analysis

The data analysis in this study was conducted using the R program (R Core Team, 2022). To assess the influence of crop type (AF-crop, UVS, MC) on the measured variables, Generalized Linear Mixed Models (GLMMs) following a Gaussian distribution were employed. The block variable was incorporated as a random effect in these models.

In cases where significant effects were observed among the measured variables, a Tukey Honestly Significant Difference (HSD) test was performed to identify which specific modality differed significantly from the others. The rejection limit for statistical significance was set at $\alpha = 5\%$.

1



2



3



4



Image 1: Sampling of *Robinia pseudoacacia* roots with Isabelle Bertrand.

Image 2: Quadrats for crop sampling.

Image 3: ^{15}N labelling showing the spraying system and labelling area (big quadrat).

Image 4: Soil sampling using hammer and chisel due to very hard soil.

Table A Soil physical, chemical and microbiological properties according to depth (0–20, 20–50 and 50–100 cm). Data are mean values (Mean) and standard deviations (Sd) and were merged for all locations ($n = 6$). The lowercase letters indicate significant differences between the soil depths (p -value < 0.05) (Siegwart et al., 2022)

	0–20 cm			20–50 cm			50–100 cm		
	Mean	Sd		Mean	Sd		Mean	Sd	
Physical properties									
Bulk density	1.17	0.05	a	1.69	0.01	b	1.87	0.10	c
Bulk density on fine particles	1.01	0.15	a	1.56	0.10	b	1.63	0.08	b
Stones volumetric proportion	14	9	a	20	14	a	47	19	b
Clay content	192.00	18.59	a	271.33	75.22	a	468.78	71.19	b
Silt content	327.78	12.32	b	322.56	23.09	b	263.78	56.68	a
Sand content	480.22	18.67	c	406.11	91.14	b	267.44	24.91	a
Moisture	37	6	a	38	4	a	37	6	a
Chemical properties									
Organic C content	8.76	1.91	c	5.07	1.79	b	3.53	0.93	a
Organic C stocks	7.41	1.21	c	5.88	0.88	b	3.00	1.22	a
Total N content	0.90	0.20	b	0.68	0.10	a	0.63	0.10	a
Total P content	0.55	0.13	c	0.45	0.13	b	0.35	0.05	a
Soil C:N	9.74	0.71	c	7.25	1.68	b	5.59	1.00	a
Soil N:P	1.67	0.33	a	1.59	0.25	a	1.78	0.22	a
Soil C:P	16.19	2.74	b	11.22	1.93	a	10.04	2.37	a
Dissolved organic C content	64.24	14.11	b	40.96	14.80	a	42.94	11.31	a
Olsen P content	40.19	13.69	c	24.67	20.68	b	8.08	4.31	a
CEC	8.35	1.93	a	10.38	0.87	a	17.97	3.62	b
pH	6.82	0.64	a	7.14	0.32	ab	7.33	0.12	b
Microbiological properties									
MBC	103.40	43.30	b	60.21	20.91	a	43.31	20.97	a
MBN	7.14	3.70	a	4.04	2.97	a	3.39	3.58	a
MBC:N	18.05	10.94	a	29.62	33.51	a	19.93	12.11	a
16S	1.99×10^9	8.76×10^8	c	1.01×10^9	4.57×10^8	b	2.50×10^8	1.34×10^8	a
18S	1.79×10^9	7.05×10^8	c	7.56×10^8	1.16×10^9	b	8.78×10^7	7.23×10^7	a
16S:18S	1.12	0.29	a	2.79	1.77	b	3.69	1.81	b
C-enzymatic activity	0.34	0.09	b	0.09	0.07	a	0.03	0.02	a
N-enzymatic activity	0.31	0.14	b	0.23	0.12	ab	0.16	0.14	a
P-enzymatic activity	0.83	0.17	b	0.45	0.13	a	0.35	0.11	a
C-enzymatic specific activity	19.39	4.31	b	7.98	3.52	a	3.65	1.57	a
N-enzymatic specific activity	16.63	5.45	a	20.62	9.56	a	21.34	16.90	a

3 Results

3.1 Crop density and biomass

The plant density was significantly higher in MC than in AF (+48%)(Table 1). Tiller and total crop biomasses were also significantly higher in MC than AF (+28% and +25%, respectively) (Figure 3A). The tillering intensity, spike density and the root and spike biomasses were all not significantly different between AF and MC (Table 2, Figure 3A).

Table 1: Intensity of tillering, plant density in April 2021 and spike density in June 2021 in agroforestry (AF) and monocrop (MC). Values represent the mean and standard deviation of eight field replicates. Letters in superscript represent statistical significance between culture types. Different letters indicate significant differences, while similar letters indicate insignificant difference. The p-value indicates the probability of obtaining the observed results, assuming that the null hypothesis is true.

System	April 2021		June 2021
	Tillering intensity (nb of stalks/seed)	Plant density (nb of stalks/m ²)	Spike density (nb of spikes / m ²)
AF	1.9 (± 0.4) ^a	607 (± 135) ^a	445 (± 91) ^a
MC	3.2 (± 0.5) ^a	900 (± 198) ^b	540 (± 94) ^a
	p = 0.133	p < 0.001	p = 0.059

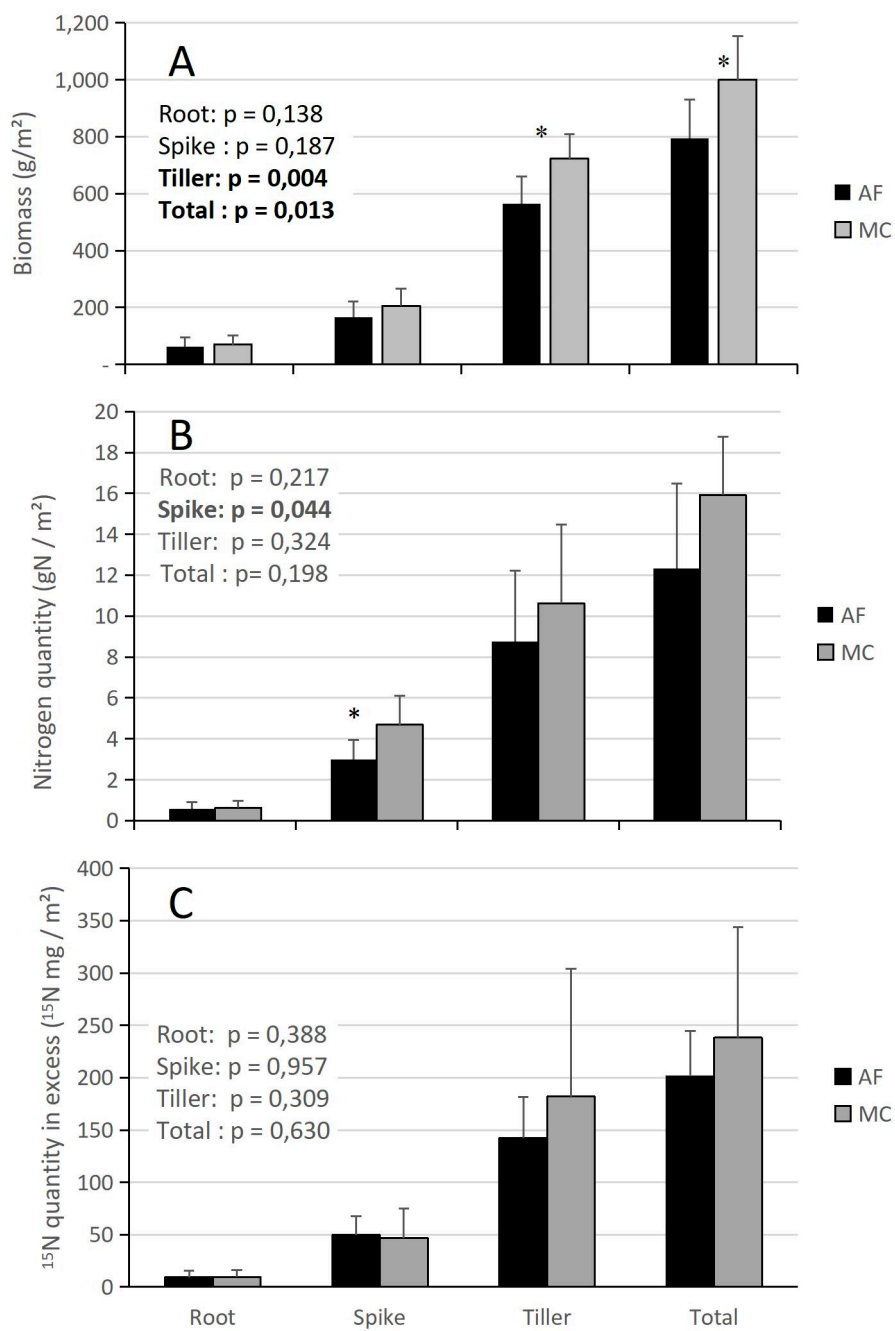


Fig. 3: Dry biomass (A), nitrogen quantity (B) and ¹⁵N quantity in excess (C) of root, tiller, spike and their summation (total) in agroforestry (AF) and monocrop (MC). Values represent the means of eight field replicates. Asterisk above the bars indicates treatments with significant effect.

3.2 Percentage of nitrogen in the compartments

The percentage of N was significantly higher in spikes of MC than in AF averaging $2.28 \pm 0.31\%$ and $1.84 \pm 0.42\%$ respectively (Table 2). The percentage of N in the tiller and in the root were lower than in spikes, averaging 1.55 ± 0.75 and $0.90 \pm 0.2\%$ respectively, and did not differ significantly between AF and MC (Table 2).

Table 2: Percentage of nitrogen in crop roots, spikes and tillers in agroforestry (AF) and monocrop (MC). Values represent the mean and standard deviation of eight field replicates. Letters in superscript represent statistical significance. Different letters indicate significant differences, while similar letters indicate insignificant difference. The p-value indicates the probability of obtaining the observed results, assuming that the null hypothesis is true.

System	%N in root	%N in spike	%N in tiller
AF	0.91 (± 0.18) ^a	1.84 (± 0.42) ^a	1.56 (± 0.60) ^a
MC	0.88 (± 0.25) ^a	2.28 (± 0.31) ^b	1.53 (± 0.72) ^a
	p = 0.442	p = 0.035	p = 0.949

3.3 Crop nitrogen quantity

The total amount of nitrogen in crops by unit of surface averaged 16 ± 3.9 g N/m², i.e., 160 ± 39 kg N ha⁻¹ in MC while in AF the average was 12 g N ha⁻¹, i.e., 120 ± 95 kg N ha⁻¹ (Figure 3B) but the difference was not significant. The amount of N in spikes was significantly higher in MC than in AF (p=0.014), while roots and tiller did not differ significantly.

3.4 Distribution of ¹⁵N excess in plant and tree parts

A total excess of ¹⁵N of 219 ± 80 mg ¹⁵N/m² was found across the treatments with no significant difference between AF and MC (Figure 3C). This quantity represented nearly half of the ¹⁵N applied. No statistically significant difference exists for roots, spike and tiller in terms of ¹⁵N quantity (Figure 3C). The percentage of ¹⁵N atom excess values did not evidence ¹⁵N that originated from the labelling in either the UVS nor the trees (Figure 4).

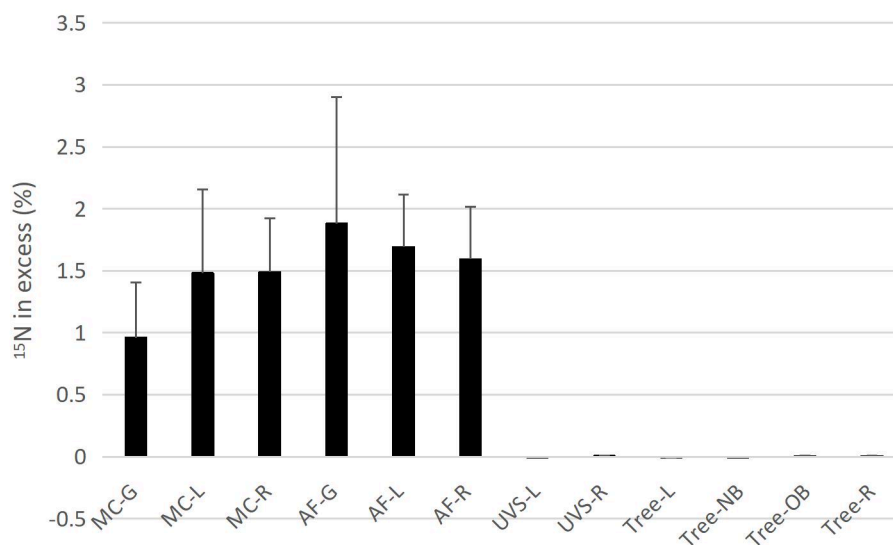


Fig. 4: ¹⁵N atoms excess % in AF-crop leaf (AF-L), AF-crop root (AF-R), AF-crop spike (AF-G), Monocrop-leaf (MC-L), Monocrop-root (MC-R), Monocrop-spike (MC-G), tree leaf (Tree-L), tree new branch (Tree-NB), tree old branch (Tree-OB), tree root (Tree-R), leaf of understory vegetation strip (UVS-L) and roots of understory vegetation strip (UVS-R). Values are means of eight field replicates.

3.5 Microbial biomass C, N and ¹⁵N excess

Insignificant difference was obtained for microbial biomass C and microbial biomass N (Table 3) between UVS, AF and MC. Due to a low level of N in the microbial biomass, C:N ratio was high in all plots, ranging from 46 to 94. Table 4 presents the %¹⁵N in excess in the extractable soil solution before and after soil fumigation. The %¹⁵N in excess in non-fumigated extracts shows the absence of ¹⁵N in the soil of the UVS and an enrichment of around 2,3% in ¹⁵N coming from the labelling in AF and MC soil. The ¹⁵N in excess in the fumigated soil extractable solutions in AF and MC were always lower than their non-fumigated counterparts, indicating an addition of ¹⁴N in the fumigated soil solution extracts linked to microbial cell lysis.

Table 3: Microbial biomass C, N and microbial biomass C:N ratio (MB C:N) in alley crop (AF), understory vegetation strip (UVS) and monocrop (MC). Values represent the mean and standard deviation of eight field replicates. Letters in superscript represent statistical significance between culture types. Different letters indicate significant differences, while similar letters indicate insignificant difference. The p-value indicates the probability of obtaining the observed results, assuming that the null hypothesis is true.

System	MBC (g/m ²)	MBN (g/m ²)	MB C:N
UVS	69.0 (± 27) ^a	0.92 (± 0.3) ^a	78.2 (± 38.6) ^a
AF	50.2 (± 10.2) ^a	0.63 (± 0.3) ^a	94.6 (± 38.6) ^a
MC	54.2 (± 11.3) ^a	1.3 (± 0.5) ^a	46.7 (± 20.6) ^a
	p = 0,141	p = 0,157	p = 0.55

Table 4: Percentage of ^{15}N in excess in non-fumigated and fumigated soil extract from the understory vegetation strip (UVS), alley crop (AF) and monocrop (MC). Values represent the mean and standard deviation (in parenthesis) of eight field replicates. Letters in superscript represent statistical significance between culture types. Different letters indicate significant differences, while similar letters indicate insignificant difference. The p-value indicates the probability of obtaining the observed results, assuming that the null hypothesis is true.

System	% ^{15}N in excess in non-fumigated soil extract	% ^{15}N in excess in fumigated soil extract
UVS	-0.23 (± 0.37) ^b	0.0006 (± 0.005) ^b
AF	2.07 (± 0.79) ^a	1.57 (± 0.39) ^a
MC	2.50 (± 0.50) ^a	2.13 (± 0.41) ^a
	p < 0.001	p < 0.001

3.6 Soil water content

The soil water content recorded across the site, indicates generally arid conditions with soil water content at 1.9 ± 0.5 % in AF and 2.2 ± 0.9 % in the MC (no significant difference between AF and MC) and significantly higher ($p=0.022$) at 3.6 ± 0.6 % in the UVS.

4 Discussion

4.1 Impact of agroforestry on crop biomass and yield

Despite the relatively young age of the site (four-year-old), the influence of different crop systems on crop development was evident, with the monocrop exhibiting higher plant density (+48%) three months after crop sowing and higher total crop biomass at harvest (+25%) compared to agroforestry. Previous studies have indicated that agroforestry can reduce the growth and yield of alley crops (Querne et al., 2017; Abbasi Surki et al., 2020; Temani et al., 2021; Ivezic et al., 2021). However, this effect is typically observed in older sites where the tree canopy is closed, resulting in significantly reduced light reaching the crop surface (Artru et al., 2017). In Mediterranean regions, the typical range of yield reduction in alley crops due to agroforestry falls between -10% and -30% (Dufour et al., 2013). However, higher reductions have been reported depending on the specific location and system (Li et al., 2008; Artru et al., 2017). Agroforestry has also been found to cause significant reductions in tiller and spike numbers of the associated crop (Kohli and Saini, 2003; Gill et al., 2009; Kaur et al., 2010; Dufour et al., 2013; Inurreta-Aguirre et al., 2018; Slafer et al., 2022).

In our study, we noted a significant reduction in aboveground biomass at harvest in barley under agroforestry compared to the monocrop. However, the reduction in root biomass was found to be insignificant. This contrast in

yield output between agroforestry and the monocrop was further confirmed by the overall yield estimate for the site, obtained by harvesting a 1.5-meter-wide, 10-meter-long strip in the center of the alley and in the monocrops on June 30, 2021, which showed an 85% increase in yield for the monocrop (Marion Forest personal communication, results not shown). Additionally, during this harvest, no significant differences in crop yields between the edge of the tree row and the centre of the agroforestry alley was observed (results not shown).

In contrast, a study conducted on the same site one year earlier, focusing on wheat, showed no significant difference in aerial biomass during the harvest stage. However, it did reveal a notable decrease in root density in wheat plants located 1 meter from the tree row compared to those situated 4 meters away, particularly within the 0-20 cm soil depth (Siegwart et al., 2022). These discrepancies between the two years can be attributed to the inherent differences between wheat and barley, as well as the influence of climate conditions on growth and the root-to-shoot ratio (Boudiar et al., 2020). Notably, the year 2020 experienced drier conditions compared to 2021, with cumulative rainfall totalling 89mm and 119mm from January to the beginning of June, respectively. The decrease in crop biomass observed in agroforestry compared to the monocrop is often attributed to competition for water and nutrients from the trees. Previous studies (Gao et al., 2013; Isaac and Borden, 2019; Ivezic et al., 2021) have suggested that black locusts,

with their wide-spreading root system (Huntley, 1990) and high transpiration rate (Mebrahtu and Hanove, 1991), may contribute to increased water loss from the soil near the crop zone. However, this effect is typically seen only when the black locusts on the site have reached an appropriate age to exert this influence. In agroforestry systems with understory herbaceous strips (UVS), which have extensive vertical and horizontal root systems (Skinner and Comas, 2010; Sainju et al., 2017; Oerter et al., 2021), competition for water and nutrients could also be attributed to the UVS or to the combined effect of the tree and the UVS. Recent studies have emphasized the significant role of the UVS in influencing soil properties, microbial activity, and fauna (Udawatta et al., 2008; Bambrick et al., 2010; Wotherspoon et al., 2014; Cardinael et al., 2015; Battie-Laclau et al., 2020; Guillot et al., 2021; D'Hervilly et al., 2021; D'Hervilly et al., 2022). However, extensive root mapping conducted on our site by Stiegwart et al. (2022) revealed the absence of roots from understory herbaceous strips (UVS) or tree roots within the crop zone. So in our system, if root competition between trees and UVS had occurred, it would more likely have been mediated via a mycorrhizal network rather than by direct root to root interactions, as suggested by the results of Battie- Laclau et al., 2020.

Another explanation of the difference in biomass between agroforestry and the monocrop could be due to the unequal application of the fertiliser in the

agroforestry plots. Indeed, Stiegwart et al. (2022) observed that points located 1 meter away from the tree line received a lower quantity of fertilizer compared to those located 4 meters away. This disparity in fertilizer distribution may have significantly impacted the results of our study, particularly considering that our sampling locations were between 1.6 and 2.70 meters from the tree line.

4.2 N distribution within and amongst plants

While most studies consistently report higher soil nitrogen levels in agroforestry systems compared to monocrops, it is crucial to acknowledge that many of these studies have primarily focused on older agroforestry sites, which may not align with the characteristics of our specific study system (Pardon et al., 2017; Guillot et al., 2021). Our findings revealed a lower nitrogen content in all of the plant's compartments in the agroforestry crop compared to the monocrop, the difference being only significant for the spike as illustrated in Figure 4. This result indicates a better ability of monocrops to benefit from available nitrogen to fill the grain. Indeed, the nitrogen content of the grain relies on two primary nitrogen sources: nitrogen assimilated into various plant organs before anthesis and nitrogen absorbed and reduced during grain filling and ripening (Cliquet et al., 1997). Despite the ample availability of soil nitrogen, studies have consistently demonstrated that the majority of nitrogen in the grain originates from the

remobilization of pre-existing reserves stored in the stalk and leaves of the tiller (Hannachi et al., 1996). Consequently, during grain filling, the increase in nitrogen content in the grain is associated with a concurrent decrease in nitrogen content in the senescing vegetative parts of the plant. The higher percentage and quantity of nitrogen in spike in monocrop compared to agroforestry shows a better nitrogen nutrition of MC compared to AF and that the remobilization of stalk nitrogen for grain filling is more efficient in MC than in AF. The low %N observed in the tillers and the root of the monocrop compared to those of AF (even though not significant) aligns with this process (Table 3).

4.3 N and C in microbial biomass

In our study, the microbial biomass nitrogen (MBN) was approximately 1 g N/m² (equivalent to 10 kg N ha⁻¹), which was nearly ten times lower than the MBN found in forest ecosystems (Rennenberg and Danneman, 2015; Maxwell et al., 2020). This difference could be attributed to the high temperatures and low soil water content commonly associated with Mediterranean environments that could limit the growth of microbial biomass (Aponte et al., 2010). Most studies have reported higher microbial biomass C and N in AF compared to MC (Kaur et al., 2000; Pereira et al., 2011; Rodrigues et al., 2015; Lepcha and Devi, 2020; Guillot et al., 2021). The deposition of litter associated with trees contributes to maintaining soil

moisture, reducing soil surface temperature, and increasing soil organic carbon stocks, thereby providing microbes with carbon and nitrogen sources (Crow et al., 2009; Matos et al., 2012; Bohm et al., 2014; Mayer et al., 2020). The black locust tree, known for its high nitrogen content (Brinks et al., 2011; Wos et al., 2020), can enhance mineral nitrogen levels in the topsoil (Keskin and Makineci, 2009; De Marco et al., 2013). The literature commonly highlights an insignificant trend in microbial carbon (C) between agroforestry and monocrop involving black locust species (Bohm et al., 2014; Kanzler et al., 2021). In our study site, we observed a higher microbial biomass C in the UVS compared to AF and MC (even though not significant) (Table 4). A high microbial biomass C in UVS could be attributed to the increased availability of resources (Saggar et al., 2001; Nunes et al., 2012). The UVS, being free from soil management changes like tillage helps to preserve soil carbon by avoiding disturbances, thus enhancing soil organic carbon stocks (Haddaway et al., 2017; Mondal et al., 2020; Hussain et al., 2021). Furthermore, the UVS exhibits higher moisture content and receives organic matter additions from tree litter, while root exudation improves the quantity and quality of plant rhizospheric resources, both of which positively influence soil microbial biomass (Fang et al., 2013; Zhang et al., 2019).

The microbial C:N ratio (MBC:MBN) in all treatments exceeded 30 (Table 3), which is considerably higher than previously reported values for this site

and many other Mediterranean agroforestry systems (Kaur et al., 2000; Battie-Laclau et al., 2020; Guillot et al., 2021; Siegwart et al., 2022). However, a study examining microbial communities' resilience and resistance to drought and heat stresses in Mediterranean agroforestry found MBC: MBN values above 35 for treatments subjected to combined heat and drought stresses (Guillot et al., 2019). In Mediterranean environments, soil microbes experience periods of drought, heat, re-wetting, and drying during summer, autumn, or spring (Gibelin and Déqué, 2003), which can alter the microbial community structure as well as carbon and nitrogen fluxes (Fierer et al., 2003; Mikha et al., 2005). Our results may indicate the presence of water or heat stress, or both, as our sampling occurred on a hot and dry summer day. Consequently, soil microbial organisms responded to these stresses by modifying their stoichiometry (Xu and Yuan, 2017). Additionally, the lower nitrogen content in the soil might have contributed to the higher C:N ratios observed.

4.4 ¹⁵N recovery and nutrient safety net

Around 50% of the applied ¹⁵N was recovered in the crop, which is a little bit higher but aligns with the findings of Rowe et al. (2005) who reported 34% of applied ¹⁵N accounted for in the first harvest. However, Lehmann et al. (2002) reported lower ¹⁵N recovery in sorghum due to competition from

15-year-old trees, which may not be applicable to our study since we examined 4-year-old nitrogen-fixing trees.

The uptake of ^{15}N applied was not observed in either the trees, understory vegetation, or microbial biomass. This could be attributed to the young age of our trees, which may have prevented them from establishing a sufficient density of roots below the labeling point, located at 1.40 to 2.90 m from the trees within the crop alley. As a result, they were unable to intercept the leaching nitrates (Rowe et al., 2001; Cadisch et al., 2004; Rowe et al., 2005). Limited tree root colonization and absence of UVS roots in the crop area observed during an in situ mapping conducted one year earlier (2020), further supports this explanation (Siegwart et al., 2022).

Another potential factor could be the retention of ^{15}N in the topsoil, which prevented its leaching to the soil depth where the tree roots extend, due to insufficient precipitation to cause leaching or due to the use of urea fertilizer which could lead to ammonia retention on soil particles (Trehan, 1996). A study examining the vertical distribution of ^{15}N in the soil 70 days after application (Rowe et al., 2005) revealed a significant amount of ^{15}N retained at or near the surface, suggesting that some of the applied ^{15}N became incorporated into soil organic matter and was thus protected from leaching. The presence of ^{15}N in non-fumigated soil extracts in our study confirms the

presence of ^{15}N in the topsoil, justifying the need to track the fate of applied ^{15}N in the plant-soil system over subsequent growing seasons.

The analysis of ^{15}N in excess in microbial biomass indicated that the nitrogen isotopic composition in the microbial biomass was more enriched in ^{14}N compared to ^{15}N ($\delta^{15}\text{N}$ in excess of fumigated extract was lower than in non-fumigated extract) in MC and AF. This suggests the preferential utilization of old organic matter present before labelling over inorganic nitrogen derived from the decomposition of the ^{15}N urea used for labelling, thereby resulting in the absence of ^{15}N in the microbial biomass. We speculate that the absence of ^{15}N in the microbial biomass could also be attributed to the timing of ^{15}N application, wherein the microbes were not nitrogen-limited during and after the application, as microbial retention of nitrogen typically occurs under nitrogen-limited conditions (Moshammer et al., 2014). Considering that our sampling took place during the summer period, the absence of ^{15}N in the microbial biomass may have been influenced by microbial activity. Microbial activity is well-documented to be significantly affected by soil moisture levels (Aponte et al., 2010), in addition to other factors such as soil chemical properties, temperature, and location (Cao et al., 2015; Borowik and Wyszowska, 2016). This observation aligns with the low soil water content recorded across the site, indicating arid conditions with soil water content of around 2%. These

findings collectively suggest a limited availability of water in the fields during our study period.

5 Conclusion

Our results show that neither the trees nor the UVS absorbed the applied ^{15}N . And also, no labelled ^{15}N was found in the microbial biomass, which nullifies the hypothesis that microbes would be a sink of fertilizer nitrogen in this agroforestry situation. The absence of ^{15}N in the tree was attributed to a lack of roots beneath the ^{15}N labelling area or to the ^{15}N retention in the soil. The absence of ^{15}N in the microbial biomass in our field experiment indicates that the microbial biomass did not serve as a nitrogen sink capable of limiting nitrogen leaching in this context. Additional sampling after several years of tree growth is needed to confirm the tree age effect on leached nitrate retention.

Acknowledgements The authors are thankful to the DIASCOPE experimental unit (INRAE Occitanie Montpellier) for the crop management at the DIAMS site.

Funding information Financial support was provided by Laboratoire Ecologie, Systématique et Evolution and by the French state aid managed by the ANR under the "Investissements d'avenir" programme with the reference ANR-16-CONV-0003 through the financial support provided by

CLAND under half-grant funding program. Financial support was also provided by Kano State government of Nigeria.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest

3 CHAPTER 3 : ENHANCED NITROGEN STOCK AND NUTRIENT SAFETY-NET IN A MEDITERRANEAN AGROFORESTRY SYSTEM

Abstract

A 2-year assessment of tree nutrient safety nets (from ^{15}N labelling experiment), nitrogen stocks, and biological nitrogen fixation (Ndfa) in a Mediterranean agroforestry was conducted. Quantities of nitrogen (N) and ^{15}N were assessed across all system components: N-fixing black locust tree, arable crops, weed plants, understory vegetation strips (UVS), rhizospheric soil, and soil microbial biomass. We hypothesized that a nutrient safety-net created by trees would become evident as a result of the leaching of nutrients and the increase in below-ground root growth during a one-year growth period. In both years, crop in agroforestry (AF) exhibited lower N stock (13-30%) compared to monoculture (MC). At the plot level, inclusion of trees and UVS within AF nearly compensated the N crop stock deficit in 2021, while in 2022 there was an increase in plot-level N stock by 62%. The Relative Nitrogen Content (RNC) was 0.97 in 2021 and 1.63 in 2022. While no ^{15}N was observed in the trees in the 1st year (70 days after labelling), about 2% of applied ^{15}N was recovered in trees 14 months after labeling. Ndfa values ranged from 52% to 68% in black locust trees, with B-values (values of isotope fractionation occurring during N_2 fixation) insignificantly influencing these estimates. Trees fixed 14 to 18 kg of N ha^{-1} . It is possible

that N fertilization in the field may be reducing N fixation in the trees leading to reduced NDFA estimates compared to other findings in black locust. Further research is needed to delve into these dynamics and explore the full potential of tree nutrient safety net in the system.

Introduction

Agroforestry systems represent multifunctional land use systems that integrate trees into agricultural crop and/or animal production that stands out as one of the most promising agricultural systems, harmonizing productivity, sustainability, and adaptability to climate change (Reynolds et al. 2007; Lin et al. 2008; Jose 2009; Lin et al. 2011). These systems offer several distinct advantages such as soil erosion mitigation, enhanced soil health and quality, nutrient cycling and carbon sequestration, biodiversity promotion and optimization of resource utilization including nitrate leaching reduction (Sierra and Nygren 2005, Sileshi et al., 2020; Zhu et al., 2020; Fahad et al., 2022; Elrys et al., 2023; Macedo et al., 2023).

Agroforestry holds the capacity to optimize nitrogen-use efficiency within the system by virtue of its capacity to elevate soil quality and nutrient absorption (Ilany et al. 2010; Patra 2013; Elrys et al., 2023). The incorporation of trees in agroforestry systems helps to prevent leaching of valuable nutrients by intercepting and utilizing them for growth and productivity which could add positive effect on overall agroecosystem health (Young, 1997; Van Noordwijk & Lusiana, 1999; Rowe et al., 2001; Bergeron et al., 2011; Veldkamp, 2023). Additionally, agroforestry is linked to the reduction of nitrogen surplus and nitrogen emissions in farming systems because certain trees, including nitrogen-fixing species, may exhibit

a lower nitrogen demand compared to that of agricultural crops (Sevel et al., 2014; Ren et al., 2023), thereby facilitating a decrease in external nitrogen inputs and the overall nitrogen surplus within the system (Ren et al., 2023). In a nitrogen-fixing system, the cultivation of nitrogen-fixing trees is frequently employed to counterbalance substantial nitrogen loss caused by crop harvesting (Nygren et al. 2000; Baier, 2023).

Stable isotopes of nitrogen (^{15}N) are commonly employed to measure atmospheric nitrogen fixation by legumes which hinges on the differences in isotopic composition between nitrogen available in the soil and atmospheric nitrogen (Rennie et al. 1978). Such differences may arise from the natural enrichment of ^{15}N (compared to atmosphere) in soil nitrogen (Amarger et al. 1979; Ledgard et al. 1984; Barthes et al., 1995), or from the controlled addition of ^{15}N -enriched fertilizer or organic material labeled with ^{15}N (Legg and Sloger 1975; Edmeades and Goh 1978; Phillips and Bennett 1978; Mahmud et al., 2022).

The role of agroforestry encompassing the UVS in terms of biomass and nitrogen stocks is not extensively studied in the literature. In this paper, a nitrogen stock and nutrient safety net (via ^{15}N labelling) assesment spanning two years (2021 and 2022) has been conducted in a mediterranean agroforestry encompassing all system components (a N-fixing tree, arable crop, weed plants in 2022, UVS, bulk soil and soil microbial biomass). We

have also estimated nitrogen fixation (percentage of nitrogen derived from the atmosphere and the fixation rate) in black locust (*Robinia pseudoacacia*) in 2021. We hypothesized that trees would absorb ^{15}N after 1 year of labelling due to increased leaching and below ground root growth and that agroforestry systems exhibit greater nitrogen stock potential compared to monoculture due to the combined contribution of aboveground and belowground biomass of the tree plus the herbaceous strips. We hypothesized that soil microbes play a significant and positive role in retaining leachable nitrate within agricultural (both AF and MC). We finally hypothesized that the UVS present in the agroforestry system's understory can serve as an effective reference plant for estimating nitrogen fixation in agroforestry systems, providing a reliable benchmark for nitrogen dynamics assessment.

MATERIAL AND METHODS:

For an in-depth description of the study area, site characteristics, soil and climate details, experimental design, ^{15}N labeling, sampling of plant and soil components, assessment of microbial biomass, expression of biomass metrics, and procedures for sample preparation for N and ^{15}N determination, as well as the calculation of microbial biomass parameters, please refer to Chapter 2. Below, I address the aspects of the materials and methods specific to the second year of the study.

Tree characteristics, cultural practices, and sampling

In 2022, tree height was on average $3.76 \pm 0.89\text{m}$ while DBH was 5.13 ± 1.44 cm. As for the principal crop species, pea (*Pisum sativum*) was sown on 20th December, 2021. Fertilizer was not applied to the crops for this growing season. Crop harvest was carried out on 9th June, 2022.

Sampling

In 2022, a single sampling campaign was conducted on 6th June encompassing trees (leaves, new and old branches, and roots), soil, as well as leaves and roots of UVS and crops. The crop, tree and soil sampling in 2022 was executed both within the areas labeled with ^{15}N in 2021 and in non-labeled areas situated 6 meters away from the labeled points. These non-labeled areas served as a reference for evaluating the ^{15}N status.

Quantity of nitrogen in tree and its compartments

Data on tree compartment biomasses (leaves, branches of the year, branches of the previous year, trunk, stump and roots) were obtained from DIAMS (estimated using allometric equation, Martin-Blangy et al., 2023 submitted). To compute the nitrogen quantity of each compartment, the compartment biomass was multiplied by its percentage of nitrogen (%N). It is worth

noting that the percentage of N in old branch was used to estimate the quantities of nitrogen in the old branches and the trunk. Additionally, the %N in fine root was used for quantifying the quantity of N in the entire root system (below ground compartment).

Estimation of Biological Nitrogen Fixation (Ndfa) in Robinia pseudoacacia

The ^{15}N natural abundance technique is the most commonly used method for estimating biological nitrogen fixation. It involves determining the isotopic $\delta^{15}\text{N}$ values of three sources: (i) the legume of interest, (ii) a non- N_2 -fixing reference plant growing within the same field as the aforementioned legume, and (iii) the isotopic $\delta^{15}\text{N}$ value of the legume of interest when relying solely on biological nitrogen fixation (BNF) as the nitrogen source for growth, which provides what is known as the "B value." This B value corrects for any isotopic discrimination during the uptake and redistribution of symbiotically fixed nitrogen (Bergersen et al., 1985; Shearer and Kohl, 1986; Marron et al., 2018).

In a study by Marron et al. (2018) aimed at characterizing the nitrogen fixation potential of black locust in a plantation using isotope methods, B-values for black locust in France were estimated. They employed two methods for estimation: (i) by growing trees on an N-free medium in controlled conditions (referred to as "Blab"), and (ii) by aligning Ndfa

(nitrogen derived from the atmosphere) calculated with the natural abundance method to that calculated with the ^{15}N dilution method in the field (referred to as "Bfield"). Both methods yielded consistent estimates of the B value, ranging between -1.4 ‰ and -3.2 ‰. In our current study, we utilized the B-values estimated by Marron et al. (2018) for assessing nitrogen fixation by black locust in our field. Some key assumptions that underpin the process of estimating NDFA in black locust trees considered in this paper are as follows:

1. The natural abundance method was selected as the most suitable approach for quantifying NDFA in the trees. It was assumed that the targeted black locust trees do not extend their roots into the crop area, thus mitigating any potential impact of nitrogen derived from fertilizer applications to the surrounding soil's ^{15}N content. Prior investigations conducted at the same site (Siegwart et al., 2022 through root mapping; Mahmud et al. 2023 (submitted) through ^{15}N labeling) have conclusively demonstrated this, revealing the absence of ^{15}N presence in both the trees and the grass strip that accompanies the crop.
2. Although there was a non-nitrogen-fixing tree in the study sites (*Gleditsia triacanthos*) that could have potentially served as a reference plant for NDFA estimation, we chose not to use it due to potential variations in the extent of soil exploration with the black locust trees. As the agroforestry practices in this study area subject the trees to tillage, fertilization, and

herbicide application, all of which may impact the isotopic composition of ^{15}N , these factors are clearly absent in the forestry site housing the non-nitrogen-fixing tree.

3. For our study, we opted to use leaves from the UVS (located at the base of the target tree) as the reference plant for tree NDFA estimation, positing that they engage with the same soil zone as the black locust tree. In alley systems of agroforestry, a cereal, weed, broadleaf or grass can be feasibly utilized as a non-fixing reference even if root depth disparities are present compared to the fixing species (Vallis et al. 1967; Rerkasem et al. 1988; Schwenke et al. 1998; Unkovich et al. 1994; Unkovich et al., 2008).

Since leaves generally act as the primary sink for recently fixed N (Domenach 1995), we used leaves to assess the symbiotic N_2 fixation by black locust trees. $\delta^{15}\text{N}$ values extracted from the sampled leaves of black locust and the UVS were subjected to analysis. The proportion of nitrogen derived from the atmosphere (NDFA) was estimated using the following equation as described by (Shearer & Kohl, 1986; Unkovich et al., 2008; Diatta et al., 2020).

$$\% \text{Ndfa} = 100 * (\delta^{15}\text{N ref} - \delta^{15}\text{Nfixing plant}) / (\delta^{15}\text{N ref} - \text{B})$$

In the above equation,

$\delta^{15}\text{N}$ ref represents the $\delta^{15}\text{N}$ level detected in the leaves of the reference plant growing in the same soil and time frame as the black locust (UVS). $\delta^{15}\text{N}$ fixing plant signifies the ^{15}N abundance of the black locust. B represents the ^{15}N abundance (‰) of the black locust, originating solely from N_2 fixation. The B values used in this study were adapted from Marron et al. (2018) as -3.2, -2.3, -1.4, and 0 for black locust at 23, 28, 40, and 52 months in France. Each respective B value was applied for the accurate calculation of NDFA.

N stocks determination

The estimation of total nitrogen stock was conducted at both the component and plot levels within the AF and MC systems. This involved a comprehensive assessment of nitrogen stock across various compartments, including crop biomass, rhizospheric soil, soil microbial biomass, the UVS, and the trees. Subsequently, these estimations were scaled up to provide an assessment at the plot level.

In both the AF and MC sites, the crop plots were standardized with dimensions of 17m in width and 100m in length, resulting in a total area of 1700m². While in the MC site, the entire area was dedicated to crops, in the AF site, some portions were allocated to represent the UVS and trees. These allocated areas were 2m in width, matching the width of the UVS, and extended 100m in length, totalling 200m². Since the AF trees were spaced 2

meters apart along the tree line, each plot within the AF system therefore contained 50 trees.

Furthermore, the Nitrogen Equivalent Ratio (RNC) was determined at the plot level for both agroforestry and monocrop systems. This ratio facilitated a comparison of nitrogen content between agroforestry and monocrop systems, offering valuable insights into the nitrogen dynamics and efficiency of each respective system.

The RNC was calculated using the formula:

$RNC = (N_{af}/N_{mc})$, where N_{af} is the nitrogen quantity in agroforestry (ie the sum of trees, crop and UVS) , and N_{mc} is the nitrogen quantity in the monoculture.

Statistical analysis

Data analysis was conducted using the R program (R Core Team, 2022). To evaluate the impact of crop type (AF-crop, UVS, MC) on the measured variables, Generalized Linear Mixed Models (GLMMs) were employed, assuming a Gaussian distribution. The block variable was included as a random effect in these models.

In instances where significant effects were detected among the measured variables, a Tukey Honestly Significant Difference (HSD) test was carried

out to pinpoint specific modalities that exhibited significant differences compared to the others. The threshold for statistical significance was set at $\alpha = 5\%$.

Results

¹⁵N excess in compartments

In 2021, the AF and MC systems generally exhibited a significantly higher percentage of ¹⁵N excess (between 1 and 2%) compared to the UVS and tree compartments where no ¹⁵N in excess was detected (Figure 2A). In 2022, lower but positive ¹⁵N excess was obtained across all cultures, including the tree and UVS (Figure 2B).

In 2021, the percentage of ¹⁵N in excess in the soil of AF and MC was around 2% and 0.009% in UVS. In 2022, the percentage of ¹⁵N in excess in the soil of AF and MC was around 0.035% and 0.01% in UVS

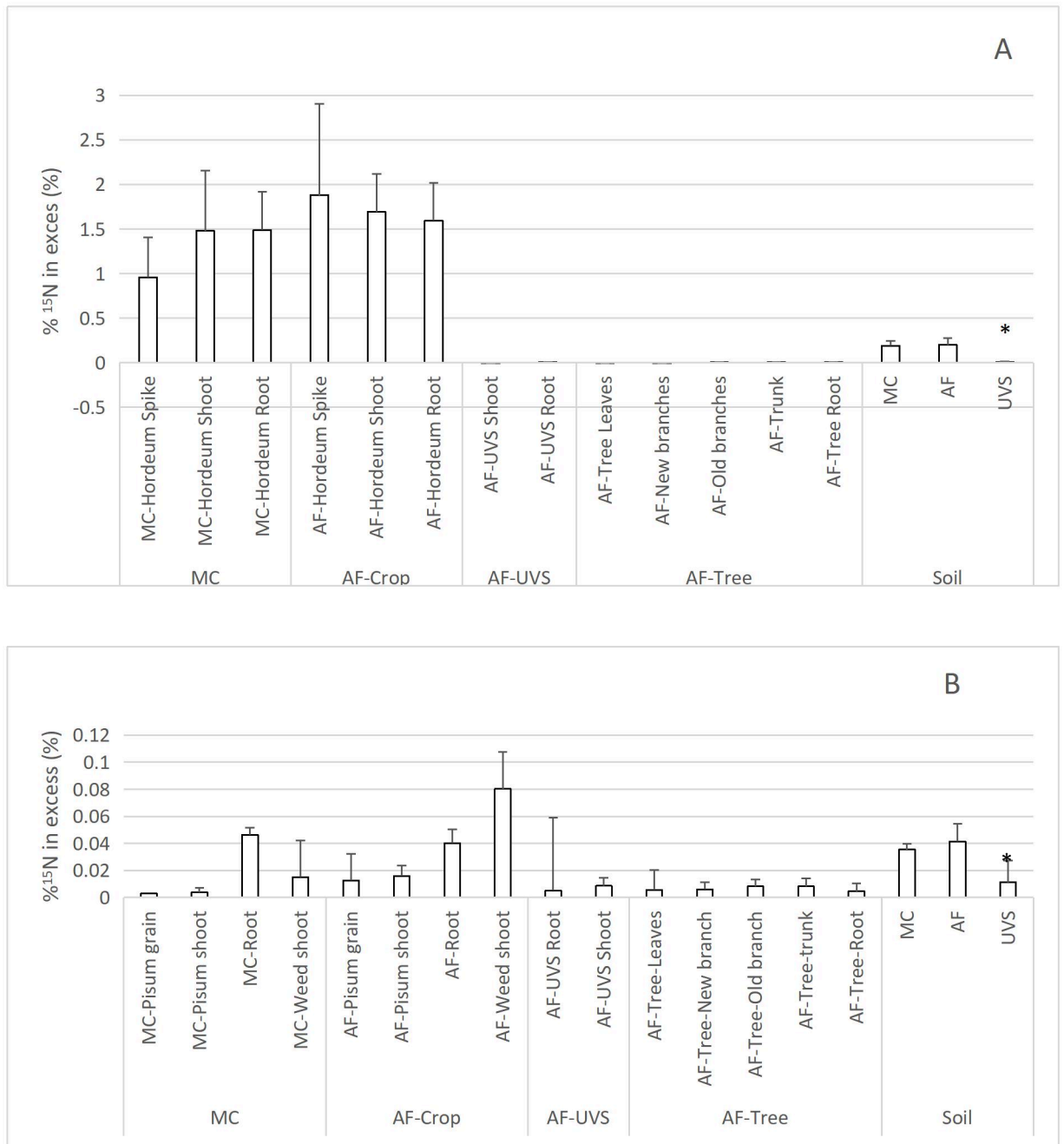


Figure 2: Percentage of ¹⁵N in excess in different compartments of monocrop (MC), agroforestry crop (AF-Crop), understory vegetation strip (UVS) and tree (AF-tree) in 2021 (A) and 2022 (B). Values are means and standard deviations of eight field replicates. The star above the bar indicates treatment that was significantly different between MC and AF in crop and between MC; AF and UVS in soil

¹⁵N quantity in excess

In 2021, 460 mg/m² of ¹⁵N was applied to the soil. Two months later, 47% of the initially applied ¹⁵N was incorporated into the crops in both the AF and MC systems (Figure 3A), while roughly 40% of it was retained within the rhizospheric soil with none retained in the microbial biomass (BM). The remaining 13% of the ¹⁵N could not be tracked within the crops, soil, or the BM. In 2021, MC and AF had similar levels of ¹⁵N in excess in their respective compartments, which is the reverse of the case in 2022, where AF had higher levels than MC. In 2022, very low level of ¹⁵N was detected in the BM in the order of 9 ± 8 ng ¹⁵N in excess/g dry soil of AF and MC. The most important storage compartment for ¹⁵N was the soil in 2022 (Figure 3B) with AF-crop and MC soils having between 34.4 and 37.7 mg ¹⁵N in excess/m², and UVS 11.7 mg ¹⁵N /m², respectively.

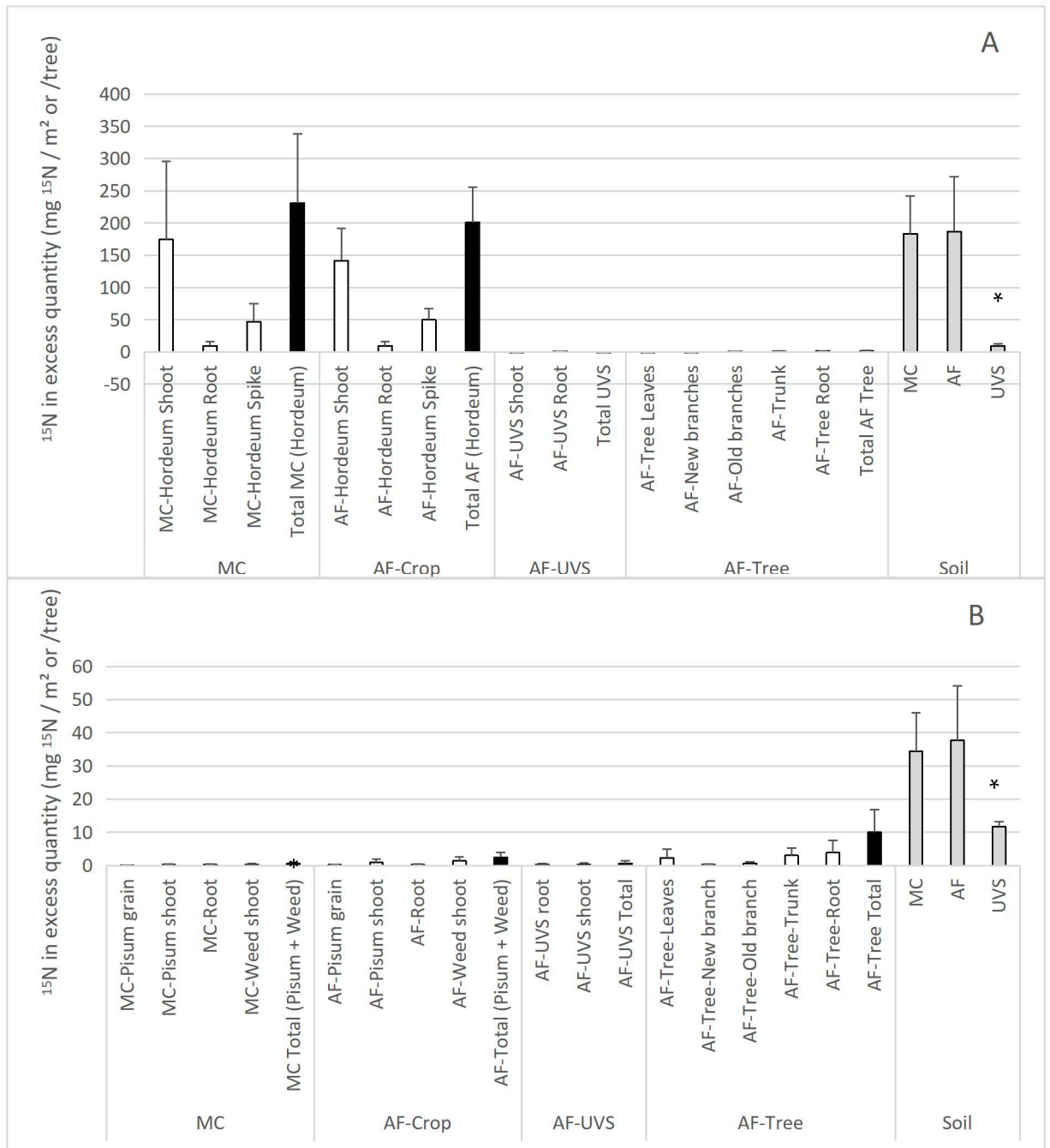


Figure 3: Quantity of ^{15}N in excess in different compartments of monocrop (MC), agroforestry crop (AF-Crop), understory vegetation strip (UVS), tree (AF-tree) and rhizospheric soil in 2021 (A) and 2022 (B). Values are means and standard deviations of eight field replicates. The star above the bar indicates treatment that was significantly different between MC and AF in crop and between MC; AF and UVS in soil.

In 2022, the AF-Crop, MC and tree systems had values of 0.5 mg/m², 2.5 mg/m² , and 10 mg tree⁻¹ of ¹⁵N in excess respectively, confirming the uptake of ¹⁵N by the trees which is in contrast to 2021 where no ¹⁵N uptake was observed in the tree and UVS (Figure 3B). ¹⁵N was distributed across all compartments of the tree, including the leaf (2.3 mg tree⁻¹), old branch (0.6 mg tree⁻¹), new branch (0.1 mg tree⁻¹), trunk (3.0 mg tree⁻¹), and roots (3.8 mg tree⁻¹) (Figure 3B). UVS also exhibited uptake of ¹⁵N , with 0.6 mg of ¹⁵N /m² in the root and shoots.

Nitrogen quantity

In 2021, the tree system stands out with the highest total nitrogen content (136.7 g tree⁻¹), driven by significant amounts in the leaf (36.15 g tree⁻¹) and root (53.41 g tree⁻¹) compartments (Figure 4A). Both MC (15.91 g/m²) and AF (12.27 g/m²) show relatively higher total nitrogen quantities compared to UVS with 4.8 g/m², which has the lowest total nitrogen content, with less nitrogen in both shoot (4.37 g/m²) and root compartments (0.44 g/m²).

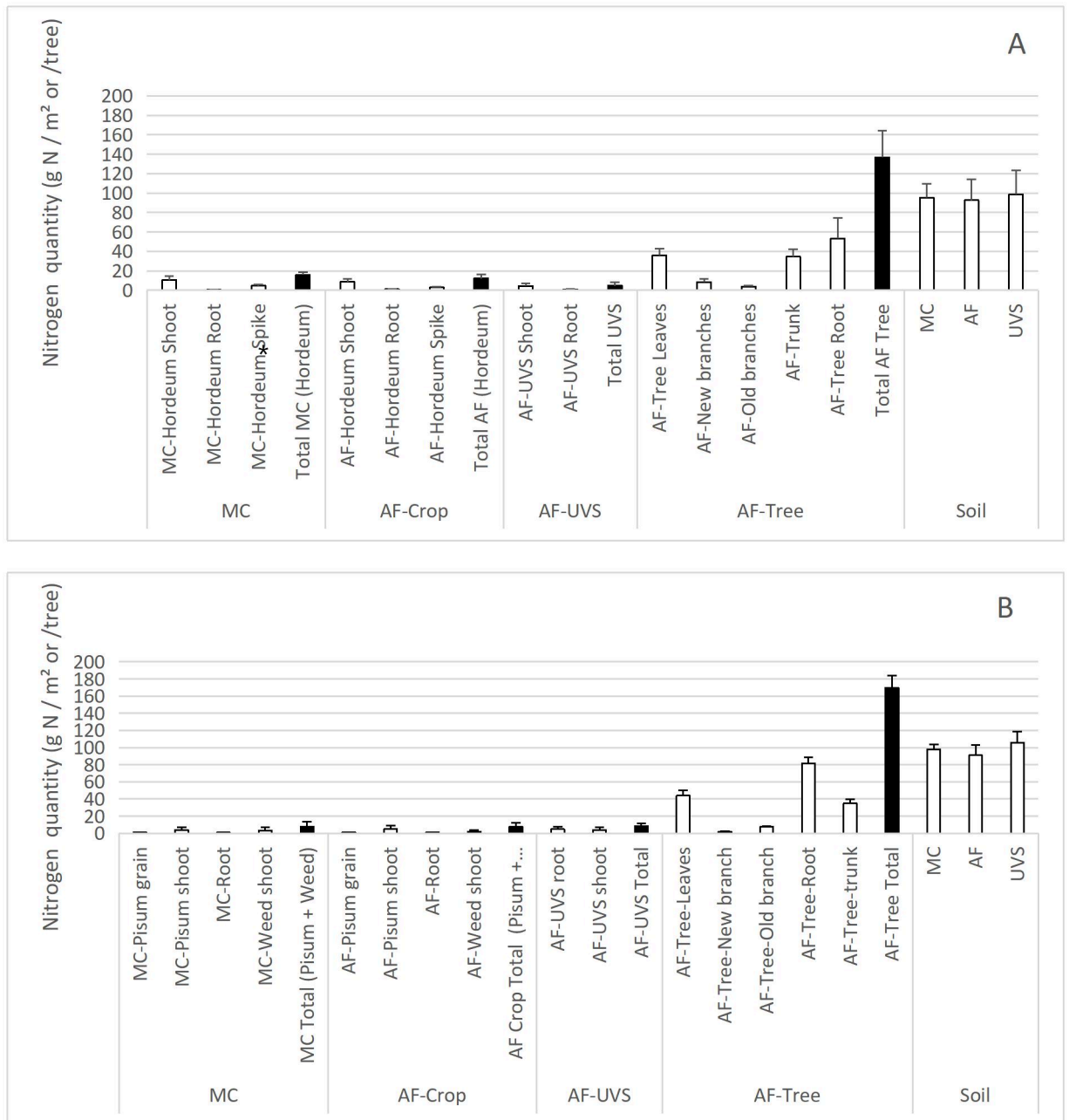


Figure 4: Quantity nitrogen in different compartments of monocrop (MC), agroforestry crop (AF-Crop), understory vegetation strip (UVS), tree (AF-tree) and rhizospheric soil in 2021 (A) and 2022 (B) expressed in g N/m² or in g N/tree. Values are means and standard deviations of eight field replicates. The star above the bar indicates treatment that was significantly different between MC and AF.

In 2022, nitrogen distribution in the AF and MC includes *Pisum* and weed. In the MC, *Pisum* grain contains 0.40 g N/m², while the *Pisum* shoot has around 3.96 g N/m², with the root containing 0.24 g N/m². The weed shoot in the MC contains 3.33 g N/m² of nitrogen. In the AF, *Pisum* grain, shoot and root contains 0.48, 4.88 and 0.36 g N/m² of nitrogen respectively. The weed shoot in the AF system contains approximately 2.08 g N/m². Similar to what was observed in 2021, the tree system stands out with the highest total nitrogen content (169.89 g N tree⁻¹), driven by significant amounts in both the leaf (43.95 g N tree⁻¹) and root (81.75 g N/m²) compartments. Both MC (7.9 g N/m²) and AF (7.8 gN/m²) systems show relatively lower total nitrogen quantities compared to UVS (8.63 g/m²) and tree (Figure 3B).

The soil nitrogen quantity was stable between the two years and there was no significant difference between the type of cultures.

Relative Nitrogen Content

Table 1 gives the quantity of nitrogen content at the plot level. In MC, the plot was made up to 1 hectare of crop while in AF the plot configuration constitutes an alley crop of 15m width and a UVS of 2m width scaled up to a hectare of AF land. Addition of trees and understory vegetation strip in agroforestry system had a notable impact on the total nitrogen content when compared to monoculture. In 2021, MC crop and AF crop respectively stored 159 and 108 kg N ha⁻¹ showing that MC surpassed AF (p=0.007) by

more than 50% due to large differences in crop biomass / m² between MC and AF (Result not shown, Mahmud et al., submitted). However, UVS and tree increased the total nitrogen content in the AF plot to 154 kg N ha⁻¹ compared to 159 kg N ha⁻¹ in MC bringing the RNC to 0.93 thereby compensating the differences brought by crop biomass differences.

When considering the soil compartment, in 2021, we observe that the MC crop-soil contains 952.9 kg N ha⁻¹, while the agroforestry system had 818.3 kg N ha⁻¹ (no significant difference). In the UVS-soil AF contains 116 kg N ha⁻¹. Therefore, at the plot soil level, the monocrop system had 952.9 kg N ha⁻¹, while the agroforestry system had 934.3 kg N ha⁻¹.

Table 1: Monocrop (MC) and agroforestry (AF) plot level nitrogen stock in different compartments and system Relative Nitrogen Content(RNC) computed from total biomass i.e nitrogen in crop (for MC) and sum of nitrogen in crop, UVS and Tree (for AF). In 2021, the plot in MC comprises barley and AF comprises barley, trees, and UVS. In 2022, the plot in MC comprises *Pisum* and weed while AF comprises *Pisum*, weed, trees, and UVS. Values represent the means from eight field replicates, with standard deviations provided in parentheses. The letters in superscripts denote significant difference between AF and MC.

Year	Compartment		N quantity in MC (kg N/ha)	N quantity in AF (kg N/ha)	RNC	
2021	Plant	Crop	159.1 (28.5) ^a	108.2 (35.6) ^b	0.93	
		UVS	-	5.6 (3.8)		
		Tree	-	40.2 (8.1)		
		Plot Biomass	159.1 (28.5)	154 (36.7)		
	BM	Crop-BM	13.4 (5.4) ^a	5.5 (2.7) ^b		
		UVS-BM	-	1 (0.3)		
		Plot BM	13.4 (5.4)^a	6.6 (2.7)^b		
	Soil	Crop-Soil	952.9 (144)	818.3 (191.4)		
		UVS-Soil	-	116.0 (28.9)		
		Plot Soil	952.9 (144)	934.3 (193.6)		
	PLOT	Biomass + BM + Soil	1,125.5 (29.5)	1095.12 (197.6)		
	2022	Plant	Crop + weed	79.2 (2.7)	68.8 (39)	1.63
			UVS		10.1 (3.6)	
Tree				50 (4.1)		
Plot Biomass			79.2 (53.2)^a	129 (39.4)^b		
BM		Crop-BM	6.2 (2.1)	9.4 (5.3)		
		UVS-BM		1.3 (0.3)		
		Plot BM	6.2 (2.1)^a	10.7 (5.4)^b		
Soil		Crop-Soil	978.9 (59.5)	803.2 (105)		
		UVS-Soil		124.2 (15.4)		
		Plot Soil	978.9 (59.5)	927.4 (106.1)		
PLOT		Biomass + BM + Soil	1064.4 (80)	1067.2 (113.4)		

In 2022, when UVS and trees were integrated into the agroforestry system (Hordeum + Pisum + Weed + UVS + Tree), the total nitrogen content reached 129 kg ha⁻¹. This total was significantly higher than that of the monoculture without UVS and trees (79.2 kg ha⁻¹), thereby producing a RNC of 1.63. That means in 2022, the agroforestry system stored by 63 % more nitrogen than what would have been achieved in monocrop under similar land allocation.

The nitrogen in microbial biomass represents between 1 to 10 % of the soil nitrogen content. The majority of the microbial biomass (BM) contribution to the overall nitrogen storage was in the crop soil in both years (5 to 13 kg ha⁻¹ in 2021 and 6 to 9 kg ha⁻¹ in 2022). BM in UVS contributed with less than 1.5 kg ha⁻¹ in both years.

Percentage NDFA

As shown in table 2, the NDFA estimated for black locust tree ranged from 52 to 68%, depending on the B-value used. The size of the B-value did not significantly affect the NDFA estimation ($p=0.065$). The biological nitrogen fixation rate of the black locust trees was estimated to range from 14 to 18kg N ha⁻¹ in our agroforestry set-up (294 trees ha⁻¹)

Table 2: Percentage of Nitrogen derived from the atmosphere (NDFA) in *Robinia pseudoacacia* with UVS as the reference plant. Values are means and standard deviations of eight field replicates obtained in 2021. Values inside the parenthesis are standard deviations of the means. B-values were adapted from Marron et al., 2018. Letters in front of the parenthesis indicates statistical significance between treatments with same and different letters indicating insignificant and significant difference respectively

B VALUE	NDFA (%)	Fixation rate (kg N/ha)
-3.2	52 (6) ^a	14 (3) ^a
-2.3	56 (6) ^a	15 (3) ^a
-1.4	60 (7) ^a	16 (4) ^a
0	68 (8) ^a	18 (4) ^a

Discussion

In the first year of assessment, the presence of a nutrient safety net was not observed. Our initial hypothesis to explain that was attributed to the absence of tree roots in the crop area, relying on the results of Siegwart et al 2021 for the study site. However, further evidence shows that absence of ^{15}N in the tree would be attributed to the retention of ^{15}N in the soil compartment which is evident from the substantial amount of ^{15}N obtained in the soil in the first year. The reported low levels of rainfall between the period of labeling and sampling (cumulative rainfall was 51.5 mm) might have hindered pronounced leaching of nitrogen thereby aggravating the ^{15}N retention in the top soil. Additionally, the labeled fertilizer utilized was urea with urease inhibition coating that serves to regulate the release rate of nitrogen fertilizer, thereby maintaining a low level of ammonium nitrogen in the soil or field water. The primary purpose of this coating is to support the long-term supply of nitrogen for crop absorption, reducing ammonia loss, and enhancing nitrogen utilization efficiency (Tang et al., 2018; Tian et al., 2017).

However, in 2022, one year after labeling, evidence of uptake of applied ^{15}N in the system emerged, with the tree shown to absorb about 2% of the applied ^{15}N . This suggests that trees may have a role in mitigating nitrogen N losses, but the extent of their contribution and their potential as a nutrient

safety net must be verified through direct measurements of N leaching and gaseous N losses within the system. This observed ^{15}N uptake after 1 year of labelling might be as a result of the leaching of nutrients and the subsequent increase in below-ground root growth during a one-year growth period. While the involvement of *Robinia pseudoacacia* in the nutrient safety net in agroforestry is not extensively documented, the absorption of leachable nitrogen by other tree species is evident. For instance, ^{15}N uptake by Jujube trees from various depths ranging from 20 to 200 cm has been documented in China (Zhang et al., 2019). Also, role of trees in nutrient safety net was demonstrated through the direct assessment of leachate in a Pecan tree and cotton alley cropping system in the United States (Allen et al., 2004). A study assessing ^{15}N uptake by four-year-old shallow-rooting *Gliricidia sepium* and deep-rooting *Peltophorum dasyrrhachis* (Rowe et al., 1999) showed that one year after labeling, *Gliricidia* recovered 0.2%, and *Peltophorum* recovered 5.6% of the applied ^{15}N from a depth of 35 cm, at a distance of 2 m from the application point to the tree rows. *Gliricidia* recovered minimal ^{15}N from all depths but obtained an estimated 44-58% of its nitrogen from atmospheric N_2 -fixation. While further assessment will be necessary to gain a better understanding of the strength and efficiency of leached nitrogen capture by black locust trees, this study confirmed their potential for nitrate leaching prevention and nutrient cycling enhancement in agroforestry systems. The presence of ^{15}N in both the tree and UVS

compartments therefore confirms our initial hypothesis.

The absence of ^{15}N in microbial biomass in both the AF and MC systems contradicts our hypothesis regarding the positive role of soil microbes in retaining leachable nitrate. A recent study that investigated the involvement of soil microorganisms in the nutrient safety-net function of agroforestry systems has provided evidence supporting the contribution of subsoil microorganisms to this role (Beule et al., 2022). Microbial community composition is frequently reported to differ between topsoil and subsoil (Zhang et al., 2017; Sosa-Hernández et al., 2018), with subsoil communities being less studied compared to those in the topsoil (Naylor et al., 2022). In our study, we considered the microbial effects in the topsoil (15cm). In contrast, Beule et al. (2022) examined subsoil microbial communities down to a depth of 60cm. Their findings indicated that tree root-derived resources and root litter, which are relatively scarce in monocrop subsoils, triggered a strong positive response in the agroforestry subsoil microbial community. As Beule et al. (2022) demonstrated that the promotion of microorganisms through trees is more pronounced in the subsoil than in the topsoil, it is essential to further investigate the subsoil microbial community's role in mediating the nutrient safety-net function in Mediterranean environments.

Nitrogen stock and its implication

We demonstrated that the quantity of nitrogen stored in crop biomass and soil could have been higher in monocrop than in agroforestry due to crop biomass reduction imposed by the agroforestry system. However, the presence of tree and understory herbaceous strip (UVS) biomass in agroforestry significantly augmented the total nitrogen stock at the plot level especially in 2022, leading to a general preference for agroforestry in terms of total nitrogen storage. Indeed at the plot level, inclusion of trees and UVS within AF nearly compensated the N crop stock deficit in 2021, while in 2022 there was an increase in plot-level N stock by 63% which is clear from the Relative Nitrogen Content (RNC) that was increased from 0.97 in 2021 to 1.63 in 2022.

A recent meta-analysis encompassing 48 studies revealed that soil nitrogen stock in agroforestry significantly surpassed that in monoculture by 13%. Furthermore, it suggested that the difference between nitrogen-fixing and non-nitrogen-fixing species was not statistically significant concerning their contribution to nitrogen stock in agroforestry (Muchane et al., 2020). Another study comparing three agroforestry treatments with rubber monoculture indicated a 33% increase in nitrogen storage in agroforestry soil relative to monoculture (Li et al., 2020). Additionally, a study that investigated nitrogen stock inventory for an 11-year-old Douglas-fir/sub-

clover agroforestry system, sub-clover monoculture, and Douglas-fir plantation revealed that agroforestry stored 2% of nitrogen in aboveground biomass compared to less than 1% in monoculture (Sharrow & Ismail, 2004). It also demonstrated that nitrogen stored by trees in agroforestry significantly exceeded that stored by trees in forestry plantations, with an additional 33 kg ha⁻¹.

We show that at a soil depth of 15cm, soil nitrogen stock was insignificantly different in MC and AF. This could be linked to the young age of the AF site and to the depth of the soil investigated (0-15cm) as other researches indicated that soil nitrogen stock in agroforestry increased with depth (Sharrow & Ismail, 2004; Isaac and Borden, 2019).

In the face of climate change, high-nitrogen stock agroforestry systems could hold promise for addressing the challenges posed by progressive nitrogen limitation under elevated CO₂ (Finzi et al., 2006; Elrys et al., 2023). In fact, drought stress, a major characteristic of future climate conditions, had been shown to increase nodule biomass of black locust trees, allowing for the maintenance of biological nitrogen fixation and countering lower soil nitrogen availability. Nitrogen derived from the atmosphere (NDFA) of drought-stressed trees was sustained at relatively higher values compared to well-watered trees (Mantovani et al., 2015). Therefore, by promoting enhanced nitrogen cycling, improving soil fertility, diversifying crop

varieties, reducing reliance on synthetic fertilizers, and enhancing overall resilience to climate change, agroforestry could significantly contribute to sustainable and climate-resilient agriculture.

Nitrogen derived from the atmosphere (Ndfa)

We utilized B-values estimated in the field by Marron et al. in 2018 to estimate Ndfa in our black locust trees using the natural abundance method. Marron et al. (2018) reported Ndfa values of 76%, 67%, 59%, and 71% for trees at 23, 28, 40, and 52 months, respectively, after planting. In our study, we obtained Ndfa values for four-year-old black locust trees of 52%, 56%, 60%, and 68% at B-values corresponding to -3.2, -2.3, -1.4, and 0, respectively with insignificant difference reported for all the B-values. The sensitivity of Ndfa to B-value depended on the reliance of the fixing species on N₂ fixation with low sensitivity reported when the target legume had high nitrogen fixation capacity (Unkovich et al., 2008; Balboa & Cimpitti, 2020).

Several studies estimated Ndfa in black locust with variations among studies due to numerous factors that affect nitrogen fixation in black locust trees, including seasonal variation (Boring et al. 1984), CO₂ concentration (Feng et al. 2004), symbiotic microbes (Tian et al. 2003), and soil properties such as nutrient availability, soil moisture, soil temperature, and pH (Noh et al. 2010; Berthold 2005). Moshki and Lamersdorf (2011) showed that seed

provenance significantly affected NDFA in black locust, where seeds from four different sources in Germany indicated NDFA ranging from 0 to 53%. Mantovani et al. in 2015 estimated NDFA in a two-year-old black locust in a post-mining site and found it to be up to 91% in a higher water treatment and 83% in a lower water treatment, but the difference was insignificant. NDFA was estimated in black locust in short rotation forest in Germany and was found to range between 63% - 83% (Veste et al. 2012).

In general, our estimated NDFA was lower than that reported by Marron et al. in 2018. That might have been due to reduced nitrogen fixation in the black locust as a result of N fertilizer application in the field as atmospheric N fixation in a nitrogen-fixing legume is known to be strongly limited by nitrogen fertilization, leading to delayed nodulation and decreased nodule size and activity (Uddin et al., 2008; Cusack et al., 2009; Unkovich et al., 2010). Additionally, there could have been some differences in the rhizobial strain between our study and that of Marron et al. in 2018 as rhizobia strain is a main factor affecting the B value (Steele et al. 1983; Yoneyama et al. 1986; Zapata et al. 1987; Guimarães et al. 2008), which affects the overall quantification of BNF (Santachiara et al. 2017; van Vugt et al. 2018).

The transfer of fixed N from black locust to associated crops could have significant implications for agriculture and ecosystems. In agroforestry systems where N-fixing trees are in association with N-fixing crops, a

compelling strategy for reducing fertilizer dependency in agriculture is presented. This approach not only saves costs for farmers but also has a positive impact on the environment by reducing pollution such as nitrate leaching and supporting sustainable agricultural practices. It fosters resilience, improves soil health, and contributes to climate mitigation, including reduced greenhouse gas emissions, making it a valuable tool in the transition to more sustainable and environmentally friendly farming practices. This approach needs to be investigated further in the future.

Conclusion

In conclusion, this study revealed insights into the dynamics of nitrogen storage in a Mediterranean agroforestry system. The initial year of assessment did not exhibit evidence of ^{15}N uptake by the tree. However, in 2022, one year after labeling, such evidence emerged, with black locust trees absorbing about 2% of the applied ^{15}N .

Microbial biomass did not retain ^{15}N in both agroforestry and monocrop systems, contradicting the hypothesis regarding the positive role of soil microbes in retaining leachable nitrate. Future research should explore subsoil microbial communities' involvement in the nutrient safety-net function in Mediterranean environments.

This study demonstrated that although crop biomass reduction in agroforestry might have led to lower nitrogen storage in crops and soil

compared to monoculture, the presence of tree and understory herbaceous strips significantly increased the overall nitrogen stock at the plot level. This emphasizes the preference for agroforestry in terms of total nitrogen storage.

Further investigations are warranted to explore the transfer of fixed nitrogen from trees to associated crops, which could revolutionize agricultural practices and foster environmental sustainability in terms of reduced N fertilizer application especially due to appreciable biological N-fixation in both trees and legume crops identified in this study.

4 CHAPTER 4: RESPONSE OF BLACK WALNUT TREES TO ELEVATED CO₂ AND CLIMATE CHANGE: ASSESSMENT IN AGROFORESTRY USING HI-SAFE MODEL

Abstract

This study explores the response of Black Walnut trees to elevated CO₂ in agroforestry, using Hi-sAFe model. The Hi-sAFe model, is a process-based biophysical model designed to capture tree-crop interactions. However, the model does not currently account for the influence of elevated CO₂ on tree growth, which is a significant limitation in the context of global change because rising atmospheric CO₂ concentrations are the primary driver of global warming, and at the same time will have direct effects on plants. My research seeks to enhance the model's ability to simulate future climate scenarios by incorporating CO₂ effects on tree growth and water use efficiency. To achieve this, the methodology involved the introduction of CO₂ effects on the Light Use Efficiency (LUE) and the water use efficiency (WUE) by modifying the LUE and the WUE sub-modules in the Hi-sAFe model. Tree-crop interactions were modeled in agroforestry setting, taking into account current and future (year 2050) climate projections and CO₂ scenarios. The results revealed several preliminary findings, including the positive effects of elevated CO₂ on tree height, the limited impact on tree diameter at breast height (DBH), and the reduction of nitrate leaching under elevated CO₂ conditions. While some aspects of the CO₂ effects were

integrated, challenges related to integrating CO₂ into a very complex model and time limitations hindered their complete implementation, particularly with respect to the WUE. Further work should include verification of the model outputs, conducting sensitivity analysis, and running long-term simulations under different CO₂ and climate scenarios. These future research steps are essential to refine our understanding of agroforestry responses to elevated CO₂ and projected climate changes, with implications for sustainable agricultural practices and global climate regulation.

Climate change impact on agriculture

Over the last 160 years, atmospheric carbon dioxide (CO₂) concentrations have undergone a notable increase, rising from 270 to 419 ppm with projections indicating an increase to between 770 and 1090 ppm by the year 2100 in the highest emission scenarios studied by the IPCC (Meehl et al., 2007; Masson-Delmotte et al., 2022). According to the emission scenarios, the trajectory of global warming is predicted to accelerate, particularly within the Mediterranean region, where temperatures are projected to surpass the global average by a considerable margin (+20%) especially during the summer months where an increase of up to 50% is expected (Lionello and Scarascia, 2018). Across tropical and temperate latitudes, a decrease in precipitation is expected with projections anticipating a reduction in summer and spring rainfall of up to 30% by the latter part of the twenty-first century, particularly within Southern Europe (Meehl et al., 2007;

Forzieri et al., 2014; Vautard et al., 2014). In light of the forthcoming climate changes and the escalating levels of atmospheric CO₂, a marginal rise in overall European crop productivity is expected especially in the northern regions of Europe (Trnka et al., 2014; Webber et al., 2016; Szewczyk et al., 2018; Webber et al., 2020). Conversely, the Mediterranean region is poised to experience the most substantial declines across all crops (Garcia-Mozo et al., 2015; Potopová et al., 2017). Yield reductions of up to 70% are anticipated by the century's end, primarily attributed to rising temperatures (Asseng et al., 2015; Mistry et al., 2017; Webber et al., 2018; Feyen et al., 2020).

In the context of this thesis which focuses on the effects of agroforestry on nitrogen cycling, it is important to note that increasing atmospheric CO₂ concentrations and climate change have significant effects on nitrogen cycles (Prentice, et al., 2012; Zaehle, 2013). Model predictions suggest that, depending on the climate scenario, global agricultural N₂O emissions could increase by 24%–31% by the years 2040–2050 (Kanter et al., 2016). Moreover, if global surface temperatures were to increase by 5°C, the emission of reactive nitrogen in the form of NH₃ into the atmosphere is projected to increase from 65 Tg N yr⁻¹ in 2008 to 93 Tg N yr⁻¹ by 2100 (Fowler et al., 2015). In cropland ecosystems, elevated CO₂ levels led to a significant increase in potential nitrification by 28%. Moreover, it substantially enhanced the abundance of the bacterial amoA functional gene

by 62%. When combined with nitrogen (N) addition and increased precipitation, elevated CO₂ levels resulted in a substantial increase in potential denitrification by 116% (Gineyts and Niboyet, 2023).

Resilient Agroforestry Systems

Projections within the realm of agroforestry reveal a different trajectory than monocropping. Future climate scenarios indicate that agroforestry systems will experience a decrease in stress factors such as heat, drought, and nitrogen stress, with stabilized crop yields (Reyes et al., 2021). Gomes et al. (2020) delved into the modeling of agroforestry coffee systems, demonstrating that introducing 50% shade cover could effectively moderate mean temperatures and maintain the suitability of around 75% of the land for coffee production by the year 2050. Furthermore, Chemura et al., (2021) conducted an estimation of projected maize yields in Ethiopia for 2050. Their findings indicated that the implementation of 20% agroforestry shade could alleviate maize yield losses by approximately 11%.

Tree Responses to Elevated CO₂: Implications on biomass and Water Use Efficiency

Trees are widely recognized as key regulators of the global climate, and their collective responses to the increasing concentrations of atmospheric CO₂ have assumed a critical role in mitigating the effects of climate change (Read et al., 2009). Elevated CO₂ has been shown to stimulate tree

photosynthesis by enhancing the carboxylation rate of Rubisco and concurrently suppressing the oxygenation of Ribulose-1,5-biphosphate (RubP) (Drake et al., 1997). Exposure to elevated CO₂ concentrations has been found to result in a 31% increase in the rate of photosynthesis in light saturated leaves and a 28% boost in overall diurnal carbon assimilation (Ainsworth and Long in 2005). Plant biomass and yield tend to increase as atmospheric CO₂ concentrations increase above current levels (Jablonski et al. 2002) depending on the experimental settings, such as controlled environment closed chambers, greenhouses, open and closed field top chambers, and free-air carbon dioxide enrichment (FACE) experiments. On average, considering multiple species under non-stressful conditions, crop yields see an enhancement of 10-20% for C3 crops and 0-10% for C4 crops at a CO₂ concentration of 550 ppm (Long et al., 2004; Ainsworth & Long, 2005). Results of meta-analysis show increases in above-ground biomass at 550 ppm for trees among all functional groups, with a 28% increase in dry matter production in young trees but little to no response in mature natural forests (Norby et al. 2003; Nowak et al. 2004; Ainsworth and Long 2005; Korner et al. 2005).

Elevated CO₂ has been shown to enhance the intrinsic water use efficiency (WUE) in trees. While few studies such as Guerrieri et al. (2019) attributed the enhancement of WUE to increased photosynthetic capacity, but the most consistent responses of plants to elevated CO₂ appears to be a decrease

in stomatal conductance (g_s) (Wand et al. 1999; Wullschleger et al. 2002; Long et al., 2004; Ainsworth & Long 2005) with 22% reduction reported by Ainsworth and Rogers (2007). There was significant variability among functional groups in how g_s responded to elevated CO_2 . On average, trees, shrubs and forbs showed a lower percentage decrease in g_s compared to C3 and C4 grasses and herbaceous crops, similar to the trend reported previously for herbaceous and woody species (Nowak et al. 2004).

Incorporating CO_2 Effects for Improved Simulation of Future Climate Scenarios: Sensitivity Analysis and Enhancement of the Hi-sAFe Agroforestry Model

Although the response of plants to changes in variables such as CO_2 , water, and temperature studied individually are reasonably well established, the complex interactions of these factors and their implications for plants' contribution to global climate regulation, both in the present and the future, is only now beginning to be comprehended. Incorporating agroforestry into this context, the exploration of how trees respond to environmental factors adds further complexity of examining how trees respond to these environmental factors, particularly the rising CO_2 levels. This is due to the intertwined feedback loops and the underlying mechanisms that govern processes such as photosynthesis, tree growth and water use.

Agroforestry system models have been developed to advance our

understanding of the complex dynamics in both the current and future conditions, within agroforestry systems. Among the models, notable mentions include the HyPAR model (Mobbs et al., 1998), the Yield-Safe model (Van der Wer et al., 2007), the APSIM model (Huth et al., 2002), and the WaNuLCAS model (Van Noordwijk et al., 1999). However, it is noteworthy that, with the exception of the WaNuLCAS model, the remaining models have limitations due to their lack of comprehensive integration of light, water, and nitrogen interactions in the system. However, as a limitation for the objectives of my work, the WaNuLCAS model itself was not originally designed for temperate regions. Hi-sAFe is the only one which is a process-based, biophysical model that is designed to encompass tree-crop interactions within agroforestry systems, capturing their spatial and temporal heterogeneity (Dupraz et al., 2019). This model emulates a 3D agroforestry environment by coupling the established STICS (version 8) crop model (Brisson et al., 1998, Brisson et al., 2002) with a new tree model (sAFe-Tree), all operating on a daily time-step. While the STICS crop model takes into account the impact of CO₂ on crop growth, the Hi-sAFe model, in contrast, does not incorporate this crucial factor on tree growth. This limitation assumes particular significance when simulating the behavior of agroforestry systems under projected future climate scenarios. As such, the Hi-sAFe model's omission of the CO₂ effects on trees creates a significant limitation in its simulation capabilities.

Therefore, the objectives of my work during the thesis were to introduce CO₂ effects on photosynthesis and water use efficiency into the Hi-sAFe model and to perform a sensitivity analysis focused on examining the impact of CO₂ within the agroforestry system, utilizing the Hi-sAFe model.

Methods

Study site

The Hi-sAFe model (Dupraz et al., 2019) was used to simulate the effect of elevated CO₂ on black walnut trees in agroforestry using a experimental plots located in Southern France (Restinclières, 43.70412° N, 3.86152° E), where agroforestry, forestry, and agricultural parcels were established in 1995 and monitored by INRAE (INRAE UMR System, 2015). This particular plot was chosen for my work because it had already been used to calibrate the model. Tree line orientation was east-west in the AF system. Tree spacing was 13 m between rows and 8 m within the row (85 trees ha⁻¹) in AF. A 1-m-wide herbaceous strip along the tree lines in AF was covered with unmanaged grasses.



Black Walnut and wheat in Restinclières, France. UMR ABSys. The agroforestry site at which Hi-sAFe model was calibrated.

The Hi-sAFe model description

As noted above, Hi-sAFe simulates a 3D agroforestry environment by coupling the existing STICS (version 8) crop model with a new tree model (sAFe-Tree) on a daily time-step. The integration of STICS and sAFe-Tree occurs via three tree–crop interaction modules that govern the dynamics of light, water, and nitrogen via simple, well-established equations (see Dupraz et al., 2019). A representative scene of the agroforestry system is divided into a grid of square “cells”, each of which contains either a homogenous crop or a single tree selected from a range of tree species (five tree species:

Hybrid Walnut, Poplar, Wild Cherry, Olive and Holm Oak). The positions of trees define two areas, the cropped area (usually an “alley” if the scene is replicated), and the uncropped area close to the trees, that can have one of several understory non-crop plant species or be bare soil. Cells are vertically divided into discrete “voxels” to capture belowground soil structure, with soil properties (root density and water and nitrogen content) homogenous within a voxel. Up to five “layers” of physical soil properties can be applied, each homogenous within the layer. Soil properties for each layer include sand, silt, clay, limestone, organic matter, and stone content. The STICS crop model simulates crop growth, crop management interventions (e.g., fertilization, tillage, irrigation), 1D vertical soil fluxes of water and nitrogen, and soil organic matter processes. To integrate STICS within a 3D heterogeneous environment, Hi-sAF_e runs an instance of STICS for each crop cell (crop or UVS) in the simulated scene.

Climate and CO₂ data

The required daily climate inputs of Hi-sAF_e are identical to those of STICS: minimum and maximum air temperature, minimum and maximum relative air humidity, global radiation, precipitation, and wind speed. A daily water table depth can be provided to simulate a fluctuating water table. However, water table was switched off in my work because of its strong exaggerating effects of CO₂ responses of trees related to changes in rooting depth. Effects

of atmospheric CO₂ on crop growth is incorporated in STICS, but we are unsure at this point in time if the STICS model was properly responding to CO₂. Hi-sAFe model on the other hand uses a constant CO₂ concentration of 360 ppm irrespective of the year and period of simulation in its current configuration.

Adding CO₂ effects in the tree component of the Hi-sAFe agroforestry model

In Hi-sAFe, daily net carbon assimilation, A (kg day⁻¹) by a tree is modeled as an empirical function of light-use efficiency, LUE (kg C Mjoules⁻¹) and daily intercepted photosynthetically active radiation, PAR (Mjoules.m⁻²)

$$A = LUE * PAR \dots\dots\dots 1$$

LUE is calculated as a function of a maximum potential LUE for the species (LUE_{max}) reduced by a water stress index (W_{stress}), a nitrogen stress index (N_{stress}), temperature stress (T_{stress}) and a leaf age effect (LA):

$$LUE = LUE_{max} * W_{stress}^{\omega LUE} * T_{stress}^{\eta LUE} * N_{stress} * LA \dots\dots\dots 2$$

where ωLUE is the sensitivity of LUE to water stress (unitless), ηLUE is the sensitivity of LUE to nitrogen stress (unitless) and T_{stress} is the sensitivity of LUE to temperature stress (unitless). Water stress is calculated as the ratio of water uptake (W_{uptake}) to water demand (W_{demand}), and nitrogen stress is calculated as the ratio of total tree nitrogen content (N_{total}) to

optimum tree nitrogen content ($N_{optimum}$). Both W_{stress} and N_{stress} , therefore, range from zero (complete stress) to one (no stress). The sensitivity factors ω_{LUE} and η_{LUE} can take on any non-negative value, with zero representing no responsiveness to the respective stress index. LA is the leaf age.

CO_2 effects was added to Hi-sAFe model as a simple modifications of the Light Use Efficiency sub-model and the whole tree transpiration sub-model. We assumed that the C, N and water components of the tree model will handle the knock-on effects of these modifications on growth, allocation, water stress and N uptake.

1) Model formulation

1.a) CO_2 effects on the Light Use Efficiency model

Plant growth and photosynthesis generally have saturating responses to atmospheric CO_2 concentrations (C_a) over CO_2 concentrations between 300-800 ppm, and these can reasonably be approximated with a Michaelis–Menten equation (see Lopez et al., 2000). This CO_2 response in the Hi-sAFe model was scaled so that it takes a value of 1 when C_a is equal to the concentrations when the model was calibrated (e.g., about 370 ppm in the year 2000, and now almost 420 ppm). The following formulation was used:

$$CO_{2lue} = C_a / (K_mCO_2 + C_a) / (CO_{2calib} / (K_mCO_2 + CO_{2Calib}))$$

Where:

CO_{2lue} is the multiplicative factor to apply to the LUE equation, and is 0 when $C_a = 0$; 1 when $C_a = CO_{2calib}$; and >1 when $C_a > CO_{2calib}$

C_a is the atmospheric CO_2 concentration

K_{mCO_2} is the half saturation constant (same units as C_a)

CO_{2calib} is the C_a when the model was calibrated (same units as C_a). See Figure 1a for a couple of examples. The measure of K_m for the CO_2 response was obtained from the literature (Wood & Hannover, 1981).

1.b) CO_2 effects on whole tree transpiration model

The approach for including CO_2 effects on WUE in Hi-sAFe is described below; however, no results are shown for this due to difficulties getting the model to run with CO_2 effects taken into account on WUE and time limitations for finishing the thesis. As such, this section provides the logic and methods for including CO_2 effects on WUE in Hi-sAFe, but clearly these will need thorough testing to ensure that the model responds as foreseen.

Intrinsic water use efficiency ($iWUE = \text{net photosynthesis} / \text{stomatal conductance}$) nearly always increases in plants as C_a rises due to stomatal control of CO_2 concentrations inside the leaf (C_i) and C_a , and often is

modelled as a function of C_i/C_a . The iWUE response to changes in C_a is fairly robust based on historical trends and elevated CO_2 experiments, at least in C3 plants (e.g., Ainsworth & Long 2005, Mathias & Thomas 2021). Historically, rising C_a accounts for about 60% of the measured variation in iWUE in trees across a very wide range of conditions (Mathias & Thomas 2021). However, the part of changes in iWUE due to increasing net photosynthesis vs. stomatal closure varies greatly and is the subject of considerable debate (Mathias & Thomas 2021). The approach we used takes advantage of this iWUE response to CO_2 and formulation of the Pereira et al. (2006) model used in the tree potential transpiration module of Hi-sAFe. We assumed that the CO_2 response of LUE (above) and iWUE can be used to derive a multiplier for the Pereira model. The formula below gives a transpiration modification factor of 1 at the CO_2 concentration at which the model was calibrated. The formula assumes that the sensitivity of iWUE is constant over a reasonable range of C_a (i.e., 300-700 ppm) which seems a good first approximation (Mathias & Thomas 2021). But we can easily change this by making the sensitivity of intrinsic WUE to CO_2 (iWUEsens) a linear function of C_a instead of a constant.

Based on Pereira et al. (2006), it has been assumed that transpiration demand (mm/day) for Walnut species can be written as:

Transpiration Demand = $ETP * Leaf Area / 2.88$ for a $30m^2$ leaf area
Walnut

Where:

ETP = Penman-Monteith ETP (mm/day)

Leaf Area = tree leaf area in m²

2.88 is an empirical constant based on observations (1/m²)

This has been modified in Hi-sAFe to use the fraction of global solar radiation intercepted by the canopy as a proxy for leaf area, which are intended to help account for size, shade and shelter effects. We suggest adding the CO₂ effects on transpiration (CO₂trans) as a multiplier in this equation, giving:

Water demand = ETP * Fraction solar radiation intercepted * CO₂trans * empirical constants.

Where the empirical constants are related to the Pereira et al. (2006) model and constants to convert the fraction of global solar radiation intercepted by the canopy into leaf area equivalents.

The CO₂trans factor is derived from the above logic as follows:

$$iWUE = 1 + iWUE_{sens} * (C_a - CO_{2calib}) / CO_{2calib}$$

$$CO_{2trans} = CO_{2lue} / iWUE$$

Where:

iWUE is the intrinsic WUE

iWUE_{sens} is the sensitivity of iWUE to changes in atmospheric CO₂

concentration and has a historical value of about 1.2 (i.e., a 100% increase in C_a gives a 120% increase in $iWUE$) (Mathias & Thomas 2019).

CO_{2trans} is the multiplier for the Pereira equation. CO_{2trans} is equal to 1 when $C_a = CO_{2calib}$; and is <1 when $C_a > CO_{2calib}$ is the C_a at which the model was calibrated (see 1.a above). Note that values of C_a below 300 ppm should probably not be used with this formulation. See Figure 1b for two examples of what this looks like for a $iWUE_{sens} = 1.2$ (a reasonable value based on Mathias & Thomas, 2019) and two different CO_{2lue} responses.

Note that whole tree transpiration may increase or decrease with rising CO_2 depending on how changes in $iWUE$ interact with growing season length, VPD and increased leaf area (all of which are accounted for in the Pereira equation). This can help explain the apparent paradox that whole forest transpiration in Europe appears to be slightly increasing over time even though CO_2 concentrations are rising. This probably occurs because increasing growing season length, VPD and increased leaf area more than counterbalance improved $iWUE$ resulting from rising CO_2 concentrations (e.g., Frank et al. 2015).

2) Model calibration

2.a) CO_2 effects on the Light Use Efficiency

We worked under the assumption that we can derive the CO₂ effects on LUE from data on tree growth and photosynthesis in elevated CO₂ experiments. The number of studies providing data on the CO₂ response of walnut is very limited. We have used an experimental data from Wood & Hanover, 1981 to fit a growth response curve to elevated CO₂ of Black Walnut seedlings (*Juglans nigra*). Getting from CO₂ effects on tree growth and photosynthesis to effects on LUE is not entirely straightforward, because increases in LUE can create a positive feedback to growth by increasing leaf area and therefore light interception at low LAI (i.e., before light interception saturates as a function of LAI). However, this effect was not significant at CO₂ concentration above 700ppm as shown in the paper published by (Wood & Hanover, 1981).

2.b) CO₂ effects on whole tree transpiration

iWUE responses to CO₂ were derived from published photosynthesis and stomatal conductance responses of Walnut by Leroux et al. (1999). This information was used in the CASTANEA mechanistic forest model (Jacobs et al., 2013) to derive the parameter estimate of the iWUE response to CO₂.

3) Application in the model

The Hi-sAFe model currently runs with a constant value of atmospheric CO₂ concentration. Ideally, this should be modified to allow for a continuous rise in CO₂ concentrations over time, especially since it would

be nice to run the model for several decades. In the long term, it might be worth including the three new parameters ($KmCO_2$, CO_2calib , $iWUEsens$) in the parameter input files of public version of Hi-sAFe model if a CO_2 version of the model would be made available to users.

4) Model system description

Alley cropping of wheat with walnut trees along the tree lines was modelled. The modelled scene in Hi-sAFe is virtually replicated in every direction using toric symmetry, approximating an infinite stand without edge effects. To duplicate the experimental conditions, the model was parameterized for a main crop of durum-wheat. However, a rape-seed crop was planted in the fourth and in the 7th years. Winter-pea was planted in the 16th and in the 19th years. The strip area surrounding the trees was bare in the first three years with spontaneous vegetation, mostly grasses in the remaining years. Simulation representing current climate was run for 20 years using Hi-sAFe's default CO_2 concentration of 360ppm. Future climate was also simulated for 20 years to represent 2050 conditions with CO_2 concentration of 550ppm, temperature was increased by $+3^\circ C$ while precipitation was reduced by -10% (see below for justification of these values for future climate).

5) The simulation run

The Hi-sAFe simulation was run in R software using the package “Hisafér” (author: Kevin Wolz). The purpose was to investigate the effect of CO₂ on LUE under two distinct climate conditions: current and future. For each condition, we have executed four scenarios, as shown in table 1 below:

Table 1: Scenarios of the Hi-sAFe model run for CO₂ effect on LUE

Scenario	Abbreviation
Current Climate	CC
Current Climate + CO ₂ effect on LUE	CC+ CO ₂
Future Climate	FC
Future Climate + CO ₂ effect on LUE	FC+ CO ₂

In our study, we chose to run simulations with constant values of CO₂ (550ppm) and future climate (+3°C of temperature; -10% precipitation) because of:

- a. Comparability: Running simulations with constant future climate and CO₂ values allows us to create a baseline scenario against which we can compare the effects of changing variables. By isolating the impacts of increasing CO₂ concentration and rising temperatures, we can more precisely assess their individual and combined effects on tree growth in the future.
- b. Simplification: Constant values simplify the modeling process, making it easier to interpret and compare results. This simplification helps us establish

a clear cause-and-effect relationship between CO₂, climate, and tree growth.

c. Realistic Projections: The selection of 550 ppm CO₂ is based on realistic projections of future atmospheric CO₂ levels. This concentration represents a significant increase from current levels and aligns with scenarios outlined in climate change models and projections.

d. Representative Temperature Increase: The choice of a +3°C temperature increase reflects the expected rise in global temperatures under certain climate change scenarios. This increase is substantial enough to assess the potential impact on tree growth while remaining within the bounds of plausible future conditions.

e. Precipitation Change: The -10% reduction in precipitation allows us to easily investigate the consequences of decreased water availability, which is a critical factor affecting tree growth.

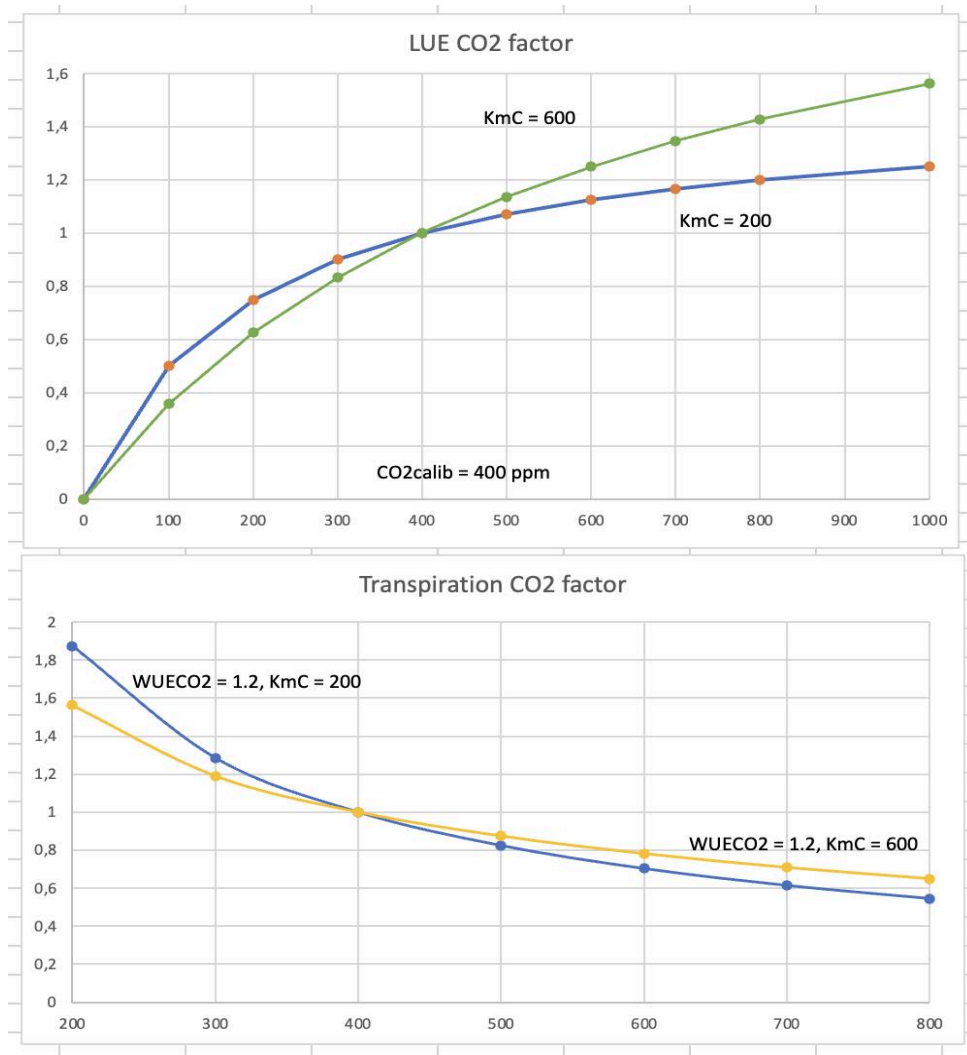


Figure 1. a) CO_{2lue} as a function of Ca , using an example assuming that the Hi-sAFe model was calibrated at 400 ppm (although 370 would be a more reasonable value based on the dates for which Hi-sAFe was calibrated at). b) CO_{2trans} as a function of Ca using a value of $iWUE_{sens} = 1.2$ and the two CO_{2lue} equations in panel a. Note that $WUECO_2 = iWUE_{sens}$ in the equations above. Note the change of scale of Ca on the transpiration factor graphic.

Preliminary results

Current climate

Tree height, DBH, rooting depth, crop yield and total crop biomass

Tree height shows a consistent increase over time. Trees were approximately 0.93 meters tall in the 2nd year, and by the 19th year, they had grown to about 4.43 meters in height. Notable periods of growth include steady height increase from 2nd to 5th years and another steady period from 16th to 20th.

The DBH of the trees shows a gradual and consistent increase over the years. DBH started at approximately 1.3 cm in the 2nd year and reached its maximum at around 34.3 cm in the 20th year. Notable periods of growth include a substantial increase in the first six years. From 11th to 15th years, DBH appears to remain relatively steady at around 28.74 cm, suggesting a period of stability in growth.

The rooting depth of the trees gradually increased over the years. It started at 0.8 meters in the 2nd year and reached 3.8 meters by the 6th year after which it remained constant at 4.2 meters (maximum soil depth) to the end of simulation period.

Crop yield and biomass

Starting from the the 2nd year of simulation, crop yield values ranges from approximately 1.14 to 3.58 t ha⁻¹, indicating a variable but generally increasing trend in production. Biomass also shows variations from year to year, ranging from around 248 to 845 kg ha⁻¹ . It follows a similar pattern to yield, suggesting that the increase in yield is associated with an increase in biomass. The 5th year of simulation stands out with a notably high yield of 3 t ha⁻¹ and a corresponding high total biomass of 845 kg ha⁻¹. There were some fluctuations in both yield and total biomass in subsequent years, but the overall trend appears to be one of increasing production in both AF and MC.

Current climate + CO₂:

Height

At elevated CO₂ concentrations, trees grew in height much faster and by the end of the simulation were 6.35 m in height compared to the 4.43 m of trees in the simulation without elevated CO₂.

DBH

Elevated CO₂ consistently produces larger tree DBH values compared to simulations without elevated CO₂ in the entire the simulation period.

Steady Growth Periods

There were two distinct periods of relative steadiness in growth in both DBH and height: One from 11th to 15th year and another from 16th to 20th year. Height also remains steady from 13th to 15th year of simulation.

Tree rooting depth

The maximum rooting depth in elevated CO₂ was attained earlier (in the 5th year) than in the scenario without elevated CO₂ (in the 7th year) suggesting faster downward growth in elevated CO₂. From 7th years onwards, rooting depth remains constant at 4.2 m for both scenarios throughout the simulation years.

Crop yield biomass

The crop yield and crop biomass were generally similar and do not exhibit a strong pattern of variability at both elevated CO₂ and the scenario without elevated CO₂.

Future climate

Tree height, DBH, rooting depth, crop yield and total crop biomass

Tree height after one year of simulation was 0.80 meters. Over the next few years, from 3rd to 6th years, there was a steady increase in tree height, with values ranging from 1.10 to 1.95 meters. The period from 7th to 10th

witnesses a more pronounced growth in tree height, with values reaching up to 3.54 meters. From eleventh to sixteenth year there was a plateau in tree height, with measurements around 3.9 meters. The years from 16th to 20th continue to show a gradual increase in tree height, albeit at a slower pace, with values ranging from 3.99 to 4.08 meters by 2014.

The baseline tree DBH starts at 0.63 cm. Over the subsequent years, from 3rd to 11th, there was a significant increase with the values raised from 2.82 to 23.71 cm during this period, indicating substantial growth in tree girth in response to changing climate conditions. However, from 11th to 15th, and from 16th to 19th, the tree DBH remains relatively stable at around 23.72 to 27.37 cm respectively. In the later years, from 13th to 20th, the tree DBH starts to increase again, with values ranging from 23.72 to 28.59 cm by the end of simulation year.

The baseline tree rooting depth started at 0.4 meters. Over the following years, from 3rd to 6th, there was a significant increase in tree rooting depth. The values rise from 1.8 to 4.2 meters during this period, indicating a substantial increase in the depth of tree roots in response to changing climate conditions. From 7th year onwards until the end of simulation year, the tree rooting depth remains consistent at 4.2 meters. This data indicates a significant increase in root depth during the early years, potentially in response to changing soil and moisture conditions.

Crop yield and total crop biomass were all similar without significant variation in the baseline scenario.

Future climate + CO₂

Tree height

There was a consistent pattern of increasing tree height for all the three scenarios, with FC+CO₂ substantially leading the growth. Tree grew faster in height in the future climate with elevated CO₂ than in future climate without elevated CO₂.

DBH

Over the subsequent years, from 3rd to 6th, all the scenarios show a consistent pattern of increasing DBH. Future climate + elevated CO₂ exhibits higher growth in DBH than the baseline. Unlike tree height, the DBH values do not appreciably differ between the future climate + CO₂ and future climate without elevated CO₂. The difference remains minimal after the initial four years of growth.

Rooting depth

In the 2nd year of simulation, tree rooting depth measurements recorded for FC and FC+CO₂ were 0.4 and 0.6 meters respectively. Over the subsequent years, from 3rd to 6th years, all the scenarios consistently maintain their

respective rooting depths without significant changes (rooting depths remain stable at 1.8 meter). From 7th to 10th, and from 10th onward to 20th years there were no observable changes in the rooting depths for any of the scenarios. Rooting depths continue to be consistent at 4.2 meters.

Crop yield

The crop yield and crop biomass were generally similar and do not exhibit a strong pattern of variability at both elevated CO₂ and the scenario without elevated CO₂. However, climate change seems to have positive effect on crop yield (see figure 8). Crop yield averaged over several years stood at 2.4 and 3.0 t ha⁻¹ for current and future climate respectively.

Nitrate leaching and denitrification

Nitrate leaching appears to be influenced by both climate and elevated CO₂, as indicated in figures 9 and 10. When averaged over several years, nitrate leaching in the current climate and future climate without elevated CO₂ amounted to 8.1 and 6.3 kg N ha⁻¹, respectively. However, in the future climate with elevated CO₂, nitrate leaching decreased further to 4.9 kg N ha⁻¹. Denitrification on the other hand was not impacted by either climate or elevated CO₂.

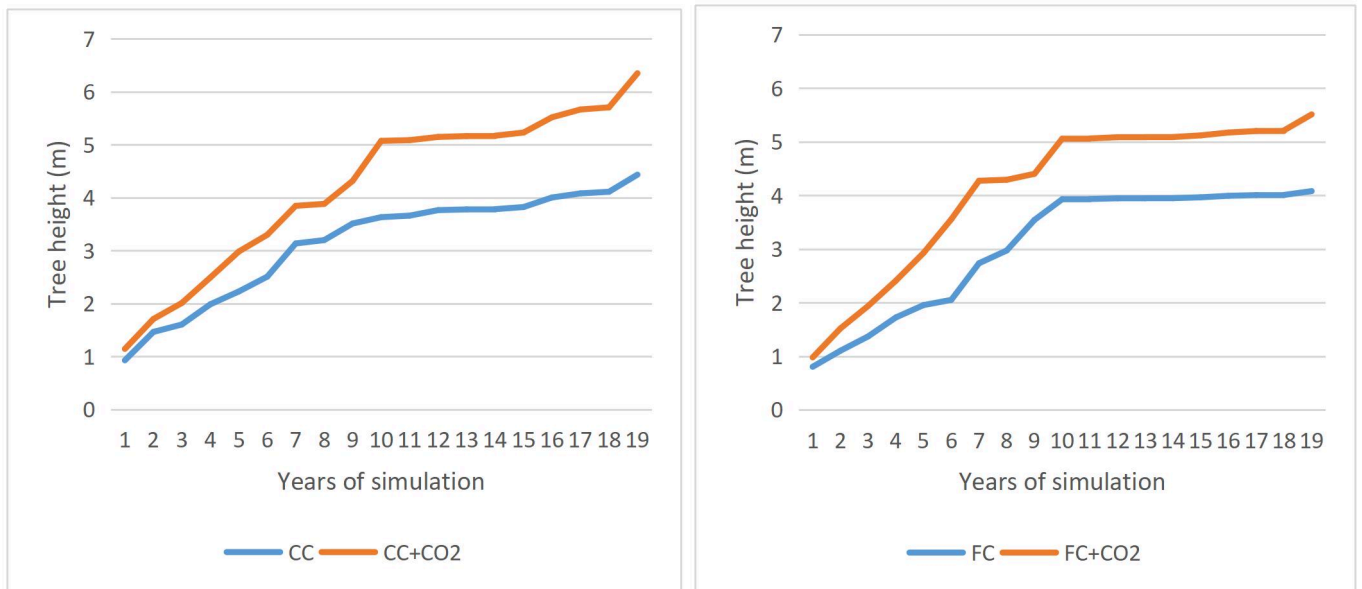


Figure 2: Twenty years' simulation of tree height under no-CO₂ (blue) and at elevated CO₂ (red) in the current and future climate conditions. CC stands for current climate while FC stands for future climate.

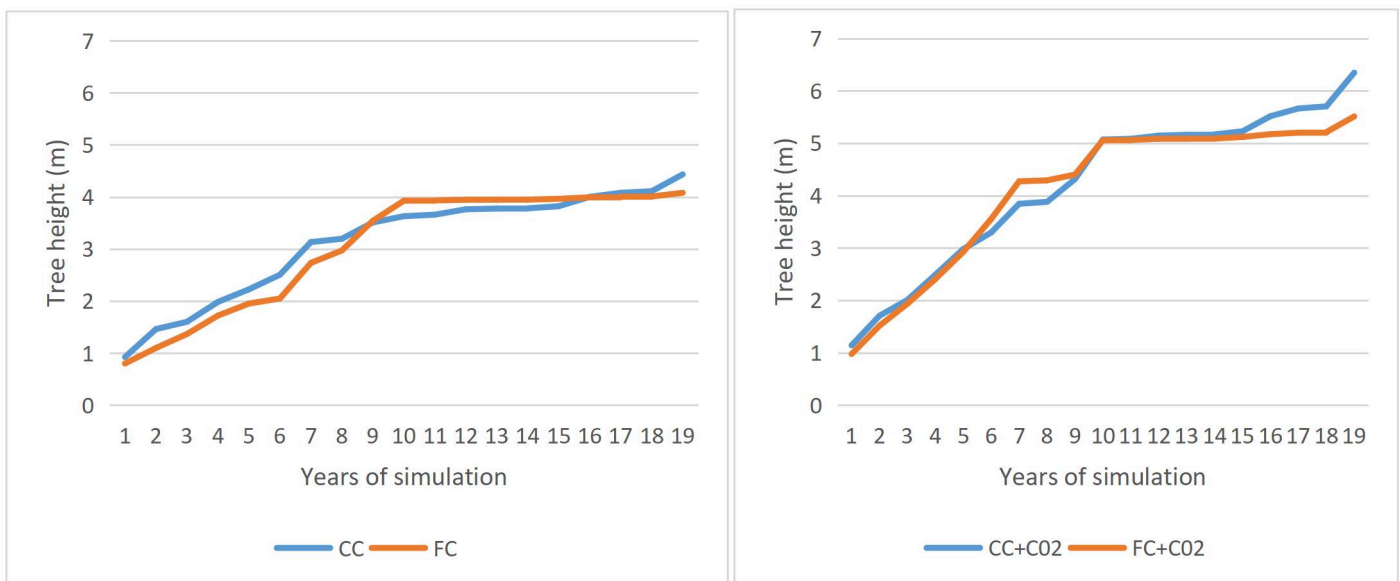


Figure 3: Comparing the effect of elevated CO₂ on height increment. CC stands for current climate; FC stands for future Climate

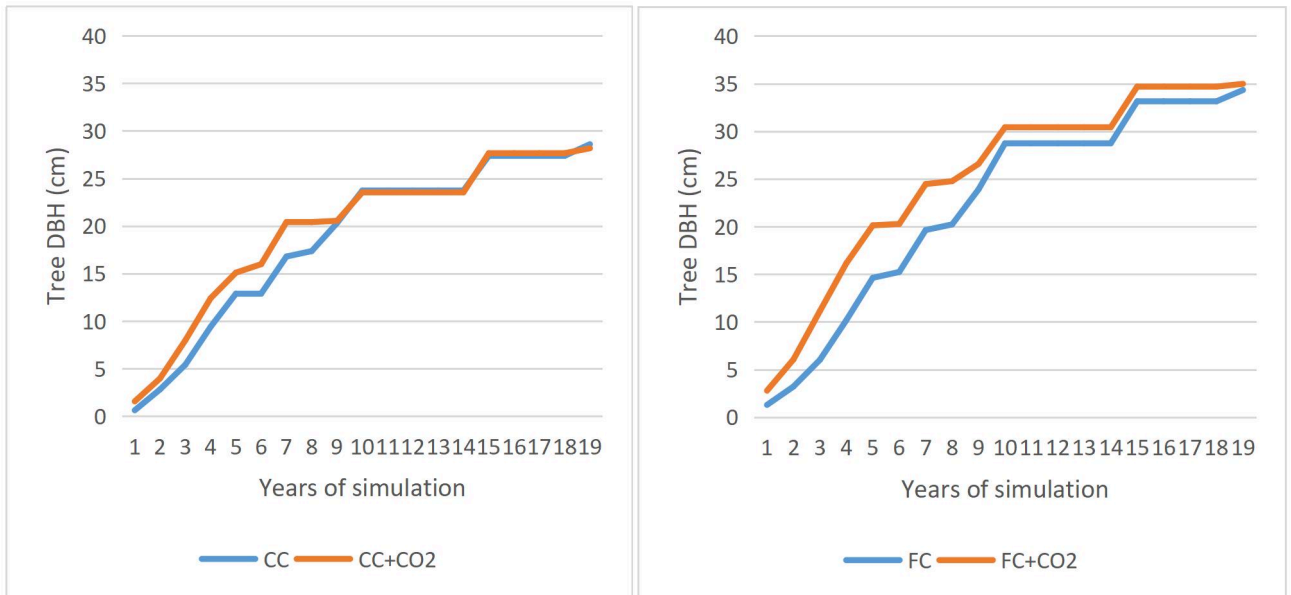


Figure 4: Twenty years' simulation of tree DBH under no-CO₂ (blue) and at elevated CO₂ (red) in the current and future climate conditions. CC stands for current climate while FC stands for future climate.

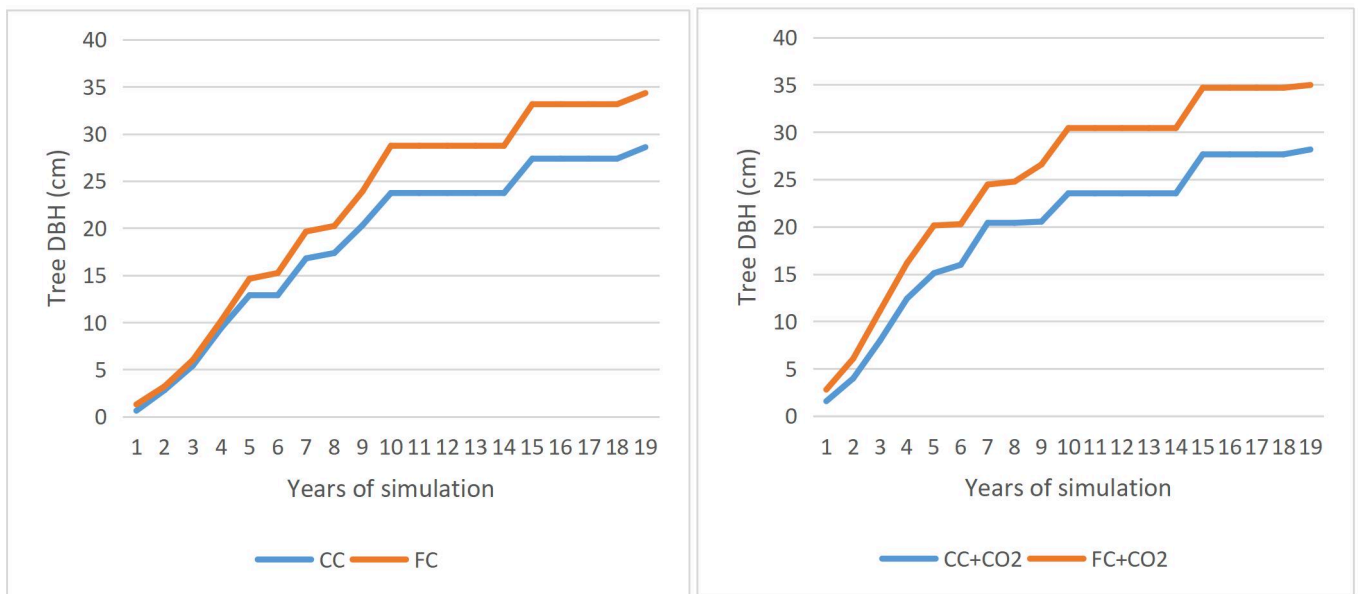


Figure 5: Comparing the effect of elevated CO₂ On DBH increment. CC stands for current climate; FC stands for future Climate

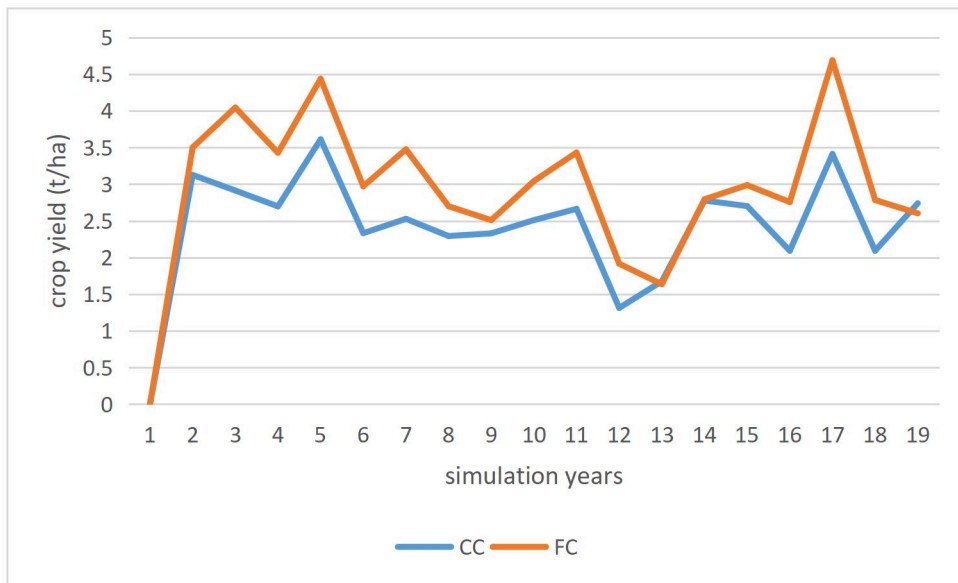


Figure 6: Crop yield in the current (CC) and future climate (FC) in agroforestry. Rape-seed crop was planted in the fourth and in the 7th years. Winter-pea was planted in the 16th and in the 19th years.

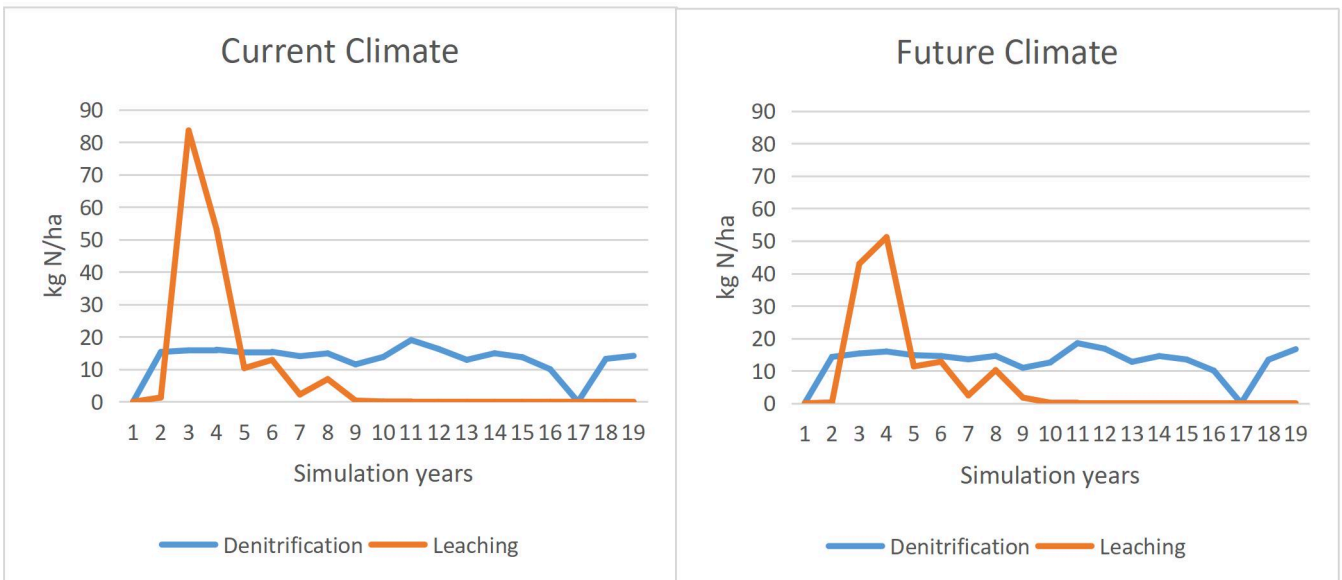


Figure 7: Nitrate leaching and denitrification in the current and future climate without elevated CO₂

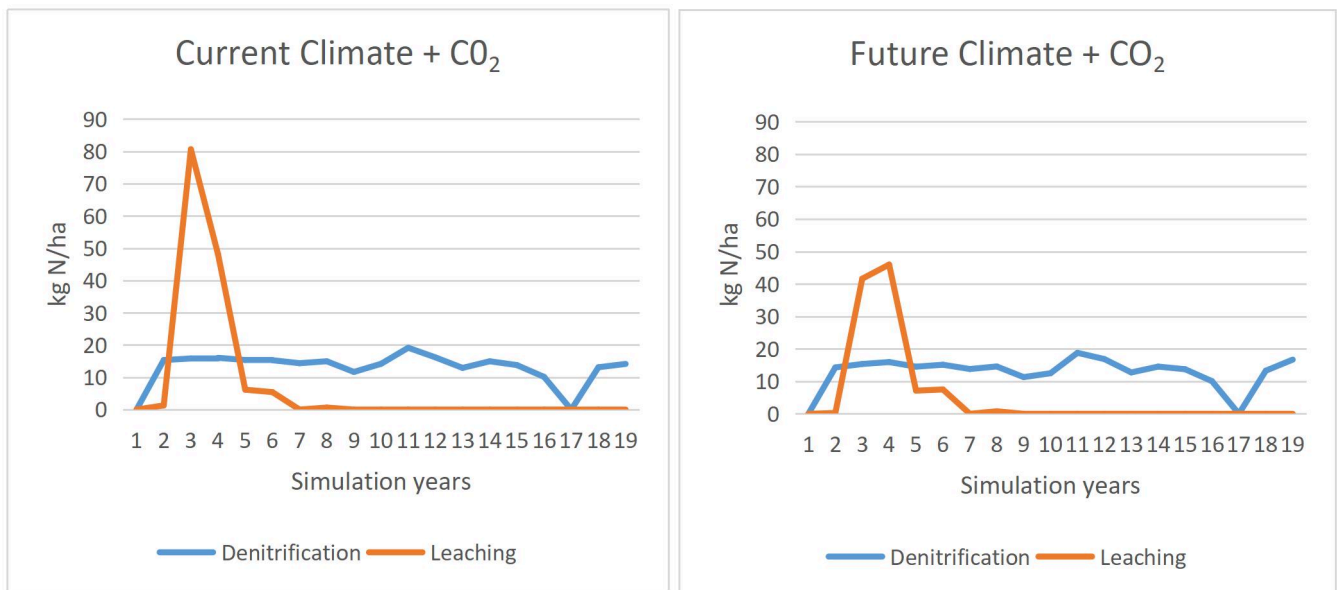


Figure 8: Nitrate leaching and denitrification in the current and future climate at elevated CO₂

Take home messages

- The first take home message is that results are very preliminary, so one need to be very cautious about their interpretation.
- Based on the model output, it appears that tree height will be positively affected by both climate change and elevated CO₂. Several factors related to climate could be contributing to this which may include temperature as warmer temperatures in the future may positively influence the rate of growth in temperate species (Dombroskie et al., 2010); precipitation (insufficient or irregular rainfall can hinder tree growth and forest succession (Gustafson et al., 2017) and CO₂ levels (increasing atmospheric CO₂ concentrations can have complex effects on tree growth). While elevated atmospheric CO₂ concentrations may stimulate photosynthesis and lead to increased growth in some cases, other factors like nutrient availability and water stress can also come into play.
- Unlike tree height, the DBH values do not appreciably differ between the future climate + CO₂ and future climate without elevated CO₂. This observation may hint at a future climatic scenario where trees attain considerable height but retain slender trunks, given the seemingly little impact of CO₂ on DBH growth. References have documented changes in height growth resulting from the combined effects of climate change

and elevated CO₂ in mature forest trees, as seen in the work of Bontemps et al. (2012). In these mature trees, the stimulation of height growth typically goes hand in hand with an increase in girth. Trouvé et al. (2015) provides some insights (in oak trees, 10-30 years old). In their research, some individual trees showed no increase in girth ($dc = 0$) but still experienced height growth ($dh > 0$). But this resulted from intense competition for light, a scenario that may not accurately represent our simulation cases.

- Comparing the different results of the model output shows that elevated CO₂ compensated the negative effect of climate change on tree growth. However, recent research reviewing trends in tree growth and intrinsic water-use efficiency in tropical regions under the influence of elevated CO₂ and climate change suggests that while rising atmospheric CO₂ enhances water-use efficiency, it doesn't necessarily stimulate radial growth in trees. This could be due to the overriding negative effects of changing climatic conditions on tree growth, which diminish the relatively small positive impact of increased atmospheric CO₂ levels (Rahman et al., 2019). Therefore, it becomes even more crucial to ensure that the Water Use Efficiency (WUE) module within our model is functioning effectively. This will enable us to gain a deeper understanding of how trees respond to these changes and their overall

effects. Climate change has a positive effect on crops, but there was no observed impact of elevated CO₂.

- Both climate change and elevated CO₂ have a reducing effect on nitrate leaching, but neither of them affects denitrification. Elevated CO₂ levels can enhance plant growth and productivity. As plants take up more nitrogen from the soil to support their growth, there is less nitrate available in the soil to leach into groundwater. Elevated CO₂ can alter soil microbial communities and reduce the activity of nitrifying bacteria, which are responsible for converting ammonium (NH₄⁺) into nitrate (NO₃⁻). When nitrification is reduced, less nitrate is produced in the soil, resulting in lower leaching. Climate change can lead to altered precipitation patterns, including decreased rainfall intensity and frequency depending on the region. This can enhance the retention of nitrate in the root zone and reduce leaching because sufficient rainfall is needed to flush nitrates deeper into the soil. We expected to see some denitrification responses, as some of the factors that affect denitrification, including warmer temperature, soil moisture, carbon, and nitrogen availability, are available under climate change and elevated CO₂. However, there was no denitrification response. It's important to understand why this was the case.

Way forward

1. Verification of the model: The current model indicates that trees grow taller instead of wider (thicker), which requires further verification to determine its alignment with real-world observations and data. It's evident from the model output that CO₂ effect on the Water Use Efficiency (WUE) is not functioning as intended (data not shown). This is a significant issue because at elevated CO₂ levels, it is generally believed that water use efficiency is enhanced in trees. Indeed, enhanced Water Use Efficiency (WUE) can have significant implications for our understanding of tree growth in the future climate for example by improving the negative effect of temperature and drought which may lead to several key effects on tree height and DBH in Future Climate. This need to be corrected and verified.

2. Sensitivity analysis: The values used for calibrating the hybrid Walnut's response to CO₂ in the modified module were primarily derived from immature Walnut species, which may not accurately reflect the characteristics of the matured Hybrid Walnut trees present in the Hi-sAFe model. This disparity could introduce uncertainty when assessing the CO₂ response. Furthermore, it has been observed that the model output suggests insensitivity of agroforestry crop yield, biomass, and denitrification to elevated CO₂ levels. Therefore, it is imperative to conduct sensitivity and

uncertainty analyses to comprehensively address these aspects and refine our understanding.

3. Experiments to run with the model: In addition to the simulations presented in Table 1, we propose conducting additional experiments involving Water Use Efficiency (WUE) under various conditions. These simulations include:

- a. WUE + Current Climate
- b. WUE + Climate Change
- c. WUE + Climate Change + Elevated CO₂

Furthermore, we recommend conducting simulations for all the scenarios previously run and comparing them with control plots, including both monocrop and forestry sites. This comprehensive approach will provide a more robust understanding of the model's behavior and its responses under different conditions.

4. Running long term simulations: Our simulations were conducted over a relatively short duration (20 years). To gain deeper insights and a more comprehensive understanding of the model's behavior, it is imperative, after completing the necessary steps of model verification and sensitivity analysis, to run long-term simulations.

These extended simulations should encompass a broader time frame and incorporate a range of CO₂ climate scenarios, including the various

Representative Concentration Pathways (RCPs) 2.6, 4.5, 6.0, and 8.5. Running simulations over longer periods will allow us to observe how the model responds to various climate scenarios over extended time horizons, providing valuable insights into its long-term dynamics and implications.

Conclusion

While significant progress has been made in integrating CO₂ effects on tree growth into the Hi-sAFe model, it is evident that further efforts are required to address existing uncertainties and refine the model. Enhancing its accuracy and applicability in the context of tree growth and climate interactions remains a paramount objective. The need for additional verification, getting the WUE module working, sensitivity analysis, and long-term simulations underscores the ongoing commitment to advancing our understanding of these complex dynamics.

5 CHAPTER 5: GENERAL DISCUSSION

5.1 SUMMARY OF KEY FINDINGS

5.1.1 Nutrient Safety Net and Tree Nitrogen Uptake

Hypothesis: The initial hypothesis suggested that trees would absorb ^{15}N after one year of labeling.

Findings: Contrary to the initial hypothesis, in the first year, the study did not find evidence of ^{15}N absorption in the tree and UVS. It was initially attributed to the absence of tree roots in the crop area and the retention of ^{15}N in the soil due to limited leaching.

Further Findings: However, in 2022 (the second year), evidence of a nutrient safety net emerged, with the tree shown to absorb about 2% of the applied ^{15}N due to increased leaching down of nitrogen and likely increase in root growth, confirming the hypothesis. This indicates that black locust trees in agroforestry systems could potentially play a role in preventing nitrate leaching and enhancing nutrient cycling especially as the tree root system develops over time.

5.1.2 Role of Soil Microbes

Hypothesis: The initial hypothesis posited that soil microbes would play a significant and positive role in retaining nitrogen and thus leachable nitrate within agricultural systems.

Findings: The study found the absence of ^{15}N in microbial biomass in both agroforestry and monoculture systems, contradicting the initial hypothesis.

Further Research Needed: The findings suggest the need for further research, especially in the subsoil, to understand the role of subsoil microbial communities in mediating the nutrient safety-net function in Mediterranean environments.

5.1.3 Nitrogen Stock in Agroforestry

Hypothesis: The initial hypothesis implied that agroforestry systems would exhibit greater nitrogen stock potential compared to monoculture due to the combined contribution of aboveground and belowground biomass of trees and herbaceous strips.

Findings: The study demonstrated that, at the plot level, the presence of trees and understory herbaceous strips (UVS) significantly augmented the total nitrogen stock at the plot level compared to monocrops in 2022.

Consistent with Hypothesis: The findings align with the hypothesis suggesting that agroforestry systems can effectively store nitrogen and contribute to sustainable agriculture, especially in the face of climate change.

5.1.4 Nitrogen Derived from the Atmosphere (NDFA)

Hypothesis: The initial hypothesis suggested that the study would find evidence of NDFA in the black locust trees.

Findings: The study estimated NDFA in black locust trees for the year 2021, with values ranging from 52% to 68%.

Practical Implications: The existence of NDFA in black locust trees in agroforestry systems is promising for reducing fertilizer dependency in agriculture, which can lead to cost savings and environmental benefits.

5.1.5 Trees response to elevated CO₂ in agroforestry

The third-year research work focused on studying the response of black walnut trees to elevated CO₂ in agroforestry using the Hi-sAFe model. The primary objectives were to introduce CO₂ effects on photosynthesis and water use efficiency into the Hi-sAFe agroforestry model. The findings and observations can be summarized as follows:

A. Incorporating CO₂ Effects: The research successfully integrated CO₂ effects into the Hi-sAFe model by modifying the Light Use Efficiency (LUE) sub-model and the whole tree transpiration sub-model (WUE). This modification was necessary because the Hi-sAFe model initially operated with a constant atmospheric CO₂ concentration, which needed to be updated to reflect the continuous rise in CO₂ concentrations over time.

B. Key Findings:

-Tree Height Response: The model's output suggested that both climate change and elevated CO₂ positively influenced tree height.

-Diameter at Breast Height (DBH): Unlike tree height, DBH did not significantly differ between future climate conditions with and without elevated CO₂.

-Nitrate Leaching: Both climate change and elevated CO₂ had a reducing effect on nitrate leaching, but they did not significantly affect denitrification. This reduction in nitrate leaching was due to increased plant growth and nutrient uptake, as well as changes in soil microbial communities.

5.2 IMPLICATIONS AND APPLICATIONS

The findings of this research hold several implications that can influence real-world applications, agricultural policies, and further research in the field of agroforestry and sustainable agriculture. Highlighted below are few of the key practical implications:

5.2.1 Nutrient Management in Agroforestry

The study's confirmation of the potential role of trees to retain leachable nitrogen (especially after confirmation in experiments with trees with better developed root systems), has practical implications for nutrient management in agroforestry. Agroforestry systems can be designed and managed to enhance nutrient retention, reducing the risk of nitrate leaching into the environment. This approach can lead to more efficient nutrient use and reduced environmental pollution. Although the study did not find evidence of a positive role of soil microbes in retaining nitrate, it highlights the

importance of studying subsoil microbial communities.

Future research can focus on understanding the subsoil microbial communities' role in nutrient cycling, potentially leading to practices that harness the microbial potential for sustainable agriculture.

Agroforestry systems, as demonstrated in the study, have the potential to enhance nitrogen stock, which might contribute to enhancing long-term soil fertility. Farmers and policymakers can consider the adoption and promotion of agroforestry practices to improve soil fertility and reduce the need for synthetic fertilizers. Agroforestry systems can contribute to sustainable agriculture by maintaining or increasing nitrogen levels.

Policymakers can consider incentivizing agroforestry practices through subsidies or other support mechanisms. Encouraging the adoption of agroforestry systems can have long-term benefits for soil fertility, reduced environmental pollution, and sustainable agriculture. Environmental policies that aim to reduce nitrate pollution in water bodies can benefit from promoting agroforestry practices as a mitigation strategy.

5.2.2 Research and Model Development

The research highlights the importance of refining models like Hi-sAF_e to accurately simulate the effects of elevated CO₂ in agroforestry systems. Continued research into model improvement can enhance their predictive capabilities. The research findings can contribute to the body of knowledge

on agroforestry, tree responses to CO₂, and climate adaptation. This knowledge can inform further studies and research directions. When the model is refined, it will help in understanding the positive effect of elevated CO₂ on tree growth which will provide agroforestry managers with valuable insights to plan for tree species that can benefit from increased CO₂ levels. The research suggests that both elevated CO₂ and climate change can reduce nitrate leaching, which is a significant source of water pollution. Agroforestry practices that consider these factors can contribute to improved water quality.

5.3 COMPARISON TO EXISTING LITERATURE

The findings of this PhD both corroborate existing knowledge and contribute to an expanding understanding of agroforestry and nutrient management. Here's an analysis of how they corroborate, challenge and expand on the existing research:

5.3.1 Corroboration with Existing Research

A. The findings of the first years corroborate existing research that suggests agroforestry systems can lead to reduced crop yields compared to monoculture systems. This phenomenon has been documented in earlier studies (e.g., Kohli and Saini, 2003; Querne et al., 2017). The extent of yield reduction (-25%) aligns with typical ranges reported in Mediterranean regions (-10% to -30%) (Dufour et al., 2013). Additionally, the findings

corroborate the notion that young trees in agroforestry systems may not have established a sufficient density of roots within the crop zone to intercept leaching nitrates. This supports previous research indicating that nutrient competition between trees and crops can depend on tree age and root density (Rowe et al., 2005).

B. The second year confirmation of trees absorbing applied ^{15}N , aligns with previous research in agroforestry systems that also shows that trees in agroforestry can play a role in reducing nitrate leaching by intercepting and retaining nutrients (Rowe et al., 1999; Allen et al., 2004; Zhang et al., 2019). The study's findings about nitrogen stock enhancement in agroforestry through the combination of tree and understory herbaceous strip biomass align with previous research indicating that agroforestry systems can store more nitrogen compared to monoculture systems (Sharrow & Ismail, 2004; Muchane et al., 2020; Li et al., 2020)

C. The study's estimation of NDFA in black locust trees is consistent with earlier studies that have reported NDFA values for different tree species (Moshki & Lamersdorf, 2011; Veste et al. 2012; Mantovani et al., 2015; Marron et al., 2018). While the specific NDFA values may vary, the principle of nitrogen fixation in agroforestry systems has been well-documented.

D. Existing research has shown that elevated CO₂ levels can stimulate tree growth by enhancing photosynthesis (Drake et al., 1997; Norby et al. 2003; Ainsworth and Long in 2004; Korner et al. 2005). The findings from the modelling work align with this knowledge by indicating that tree height is positively affected by both elevated CO₂ and climate change. This corroboration reinforces the understanding that increased atmospheric CO₂ can promote tree growth.

5.3.1 Challenging the existing research

While the findings suggest that root competition between trees and UVS may not have been a significant factor in crop yield reduction in the studied system, this contradicts some previous research indicating that competition for water and nutrients could arise from the combined effect of trees and understory vegetation (Udawatta et al., 2008; Skinner and Comas, 2010). These discrepancies highlight the complexity of agroforestry systems and the need for site-specific considerations. The study challenges the idea of a positive role of soil microbes in retaining nitrate within agroforestry systems. This contrasts with some previous research, which suggested that soil microbial communities play a significant role in nutrient cycling and retention (Beule et al., 2022).

5.3.2 Expansion on existing knowledge

A. Young Tree Impact: This study adds to the existing body of knowledge

by focusing on the effects of young nitrogen-fixing trees (4-year-old) in agroforestry systems. Previous studies have often considered the impact of older trees, and this research suggests that young trees can significantly affect nutrient competition and uptake.

B. Depth of soil analysis: The study highlights the need for further research, particularly on subsoil microbial communities. While it challenges the role of topsoil microbes, it expands our understanding of potential differences in microbial contributions at varying soil depths. The study emphasizes that soil nitrogen stock at a 15cm depth did not significantly differ between monocrop and agroforestry. This suggests that the depth of soil analysis is an important factor. While previous research may have focused on different soil depths (Sharrow & Ismail, 2004; Isaac and Borden, 2019), this study highlights the need to consider variations in nitrogen stock at varying depths.

C. Incorporating CO₂ Effects on Tree Growth in Agroforestry: The original Hi-sAFe model lacked the consideration of CO₂ effects on tree growth, and this addition to the model represents a significant novel contribution of this PhD research.

5.4 METHODOLOGICAL REFLECTIONS

The Phd research employed a range of methodologies that have both strengths and limitations. These methodologies introduce some innovative approaches that could influence future research in the field of agroforestry

and climate change. Here's a reflection on the methodologies used:

5.4.1 Strengths of the methodological approach

A. Experimental Design: The study adopted a well-structured experimental design that included both agroforestry and monocrop plots, as well as the application of ^{15}N -labeled urea. This design allowed for a controlled assessment of the impact of agroforestry on crop performance and nutrient dynamics.

B. Hypothesis Testing: The research formulated and tested specific hypotheses. This systematic approach helped in addressing specific research questions, making the study outcome-focused and hypothesis-driven.

C. Use of Isotope Labeling: The application of ^{15}N -labeled urea is a notable strength. This technique is a powerful tool for tracking nutrient movement and uptake in plant-soil systems. It allowed for precise assessment of nitrogen dynamics and allocation in the study, providing valuable insights.

D. Microbial Biomass Analysis: The study delved into microbial biomass and its isotopic composition, shedding light on the microbial community's role in nutrient cycling within agroforestry systems. This is an essential aspect for understanding the overall ecosystem functioning.

E. Site-Specific Investigation: The research accounted for site-specific factors, such as the presence of understory vegetation (UVS), and

considered the interactions between different components of the agroforestry system. This approach recognizes that agroforestry practices can vary depending on the local context.

F. Interdisciplinary Collaboration: The research involved an interdisciplinary approach, combining elements of ecology, agronomy, soil science, and microbiology. This interdisciplinary collaboration serves as a model for addressing complex agricultural and environmental challenges that require a multifaceted understanding.

5.4.2 Limitations and unanswered questions

A. Short-Term Study: The study's relatively short experimental duration of two years is a limitation. Agroforestry systems are often long-term investments, and the effects of tree-crop interactions may evolve over time. Long-term studies are required to fully understand the sustainability and economic viability of agroforestry practices.

B. Tree Age and Canopy Closure: The research focuses on young nitrogen-fixing trees (4-year-old) in the agroforestry system. Tree age and canopy closure can significantly impact nutrient competition and crop performance. Extending the study to include older trees or waiting for the canopy to close might provide more insights into long-term effects.

C. Limited Spatial Analysis: While the research considered the effects of tree proximity on nutrient distribution, it did not extensively explore the

spatial distribution of nutrient competition or root systems. Detailed spatial analyses would provide a more comprehensive view of resource competition.

D. Unequal Fertilizer Distribution: The uneven distribution of fertilizer in agroforestry plots (reported by Siegwart et al., 2022) introduced a confounding variable. Such disparities in nutrient management may not be representative of typical agroforestry practices, where uniform nutrient application is often emphasized.

E. Lack of Understory Roots: The research mentioned the absence of roots from understory herbaceous strips (UVS) within the crop zone, which raises questions about the role of UVS in nutrient competition. Further investigation into the presence or absence of understory roots would provide a more comprehensive understanding.

F. Limited Experimental Data to Parameterize Models for CO₂ response: One of the limitations is the scarcity of experimental data on the CO₂ response of black walnut trees. The reliance on a limited dataset can introduce uncertainty into the model's calibration. Future research could benefit from additional empirical data to strengthen model parameters.

G. Insensitivity of Hi-sAFe model to some parameters: The research findings indicate that agroforestry crop yield, biomass, and denitrification were insensitive to elevated CO₂ levels. The research acknowledges that the Water Use Efficiency (WUE) module in the Hi-sAFe model needs

improvement. This counterintuitive result challenges our common expectations and need to be further investigated.

H. Further Species and Diversity: The modeling research focused on black walnut trees. Future studies can expand this scope by including a broader range of tree species commonly used in agroforestry. Investigating how different tree species respond to elevated CO₂ and interact with crops can provide insights into diversifying agroforestry systems for greater resilience.

I. Mature Tree Effects: The study primarily looked at the growth response of immature black walnut trees to elevated CO₂. Future research can explore the effects of elevated CO₂ on mature trees in agroforestry systems. Mature trees may exhibit different growth patterns and interactions with crops.

J. Model Development: The Hi-sAFe model was used in this research, but model improvement and development should be ongoing. Future research can focus on enhancing the model's accuracy to better represent real-world agroforestry systems.

5.5 BROADER SIGNIFICANCE

The findings of this PhD hold significant importance in the larger context of science, society, and the world at large. Its broader significance can be understood through several key points:

A. Sustainable Development Goals: The research aligns with several United

Nations Sustainable Development Goals (SDGs), including SDG 2 (Zero Hunger), SDG 13 (Climate Action), SDG 15 (Life on Land), and SDG 17 (Partnerships for the Goals). It contributes to the global effort to achieve these goals by promoting sustainable and resilient agricultural practices.

B. Nutrient Management: Effective nutrient management is essential for sustainable agriculture and food security. Understanding nutrient dynamics in agroforestry systems is critical for optimizing nutrient use efficiency, reducing the reliance on synthetic fertilizers, and preventing nutrient leaching into the environment. This research sheds light on the complex interactions between trees, crops, and soil microbes, offering insights into nutrient cycling and retention.

C. The research findings, particularly those related to nutrient safety nets and nitrogen cycling, emphasize the potential of agroforestry in protecting natural resources and ecosystems.

D. Scientific Advancements: The research introduces modifications to the Hi-sAFe model to account for CO₂ effects. This methodological innovation can benefit future research in agroforestry and ecosystem modeling. Improved models enable scientists to make more accurate predictions and recommendations for land use and climate adaptation.

E. International Relevance: The research conducted in Southern France contributes to the international body of knowledge on agroforestry.

Agroforestry is practiced worldwide, and the insights gained from this research can be relevant to diverse geographic and climatic conditions.

F. Interdisciplinary Collaboration: Agroforestry research often involves collaboration between ecologists, agronomists, climate scientists, and other experts. This interdisciplinary approach fosters cooperation in addressing complex global challenges related to food security and climate resilience.

5.6 CONCLUSION

In conclusion, this PhD research has helped to better understand nitrogen cycling in agroforestry systems. The study confirmed the potential of a nutrient safety net in agroforestry systems, where trees can absorb and retain leachable nitrogen, offering practical implications for nutrient management in agroforestry. Agroforestry systems have been demonstrated to enhance nitrogen stock, that might provide long-term soil fertility and potentially reducing the need for synthetic fertilizers.

The research also ventured into innovative territory by integrating CO₂ effects into the Hi-sAFe model, which enhances the model's predictive capabilities for tree growth in response to climate change. The study's findings suggest that elevated CO₂ and climate change can reduce nitrate leaching, contributing to improved water quality.

Furthermore, the research both corroborates and challenges existing

knowledge in agroforestry. It corroborates the potential benefits of agroforestry in terms of nutrient retention, nitrogen stock enhancement, and the existence of nitrogen derived from the atmosphere (NDFA). However, it challenges existing beliefs regarding microbial contributions to nutrient retention and the role of young trees in affecting crop yields.

The methodologies employed in this research, from hypothesis testing to the use of isotope labeling and interdisciplinary collaboration, present strengths that contribute to the robustness of the findings. The study also highlights several unanswered questions and limitations, such as the need for longer-term research, exploration of different tree species, and model development.

The broader significance of this research is evident in its alignment with Sustainable Development Goals, its potential to improve nutrient management and protect the environment, its relevance across different geographical contexts, and its contributions to knowledge transfer, policy development, and interdisciplinary collaboration. In summary, this PhD research could have broad-reaching implications for science, society, and the world at large by advancing our understanding of agroforestry and sustainable agricultural practices.

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