Università degli Studi di Sassari

The potential of Biodiversity for Adaptation Strategies to Global Change in Mediterranean Ecosystems

Tony Chahine

A Thesis Submitted for the Degree of
Doctor of Philosophy

Doctoral Research Course in Agrometeorology and Ecophysiology of Agricultural and Forest Systems

Supervisor: Prof. Donatella Spano

Co-Supervisor: Dr. Simone Mereu

Coordinator: Prof. Severino Zara

DIPARTIMENTO DI AGRARIA

CICLO XXXV (2020-2023)

Abstract

This PhD thesis addresses the relationships between biodiversity ecosystem functionality specifically for the Mediterranean biome. While much ecological research has elucidated the role of biodiversity in controlling fundamental ecological processes, it has mainly focused on grassland ecosystems since 1990 research has started to focus on tree ecosystems as well.

Although experimental evidence clearly demonstrates a general positive relationship between biodiversity and above ground productivity, as well as other ecosystem functions, the same evidence also highlighted how the direction of the effect is also strongly dependent on the community composition and environmental context. In the last two decades, attention has does shifted towards understanding the underlying ecological mechanism that determine the outcome of mixing species.

The research mostly focused on investigating the Water Stress Gradient Hypothesis (SGH) and its implications for ecosystem responses to changing water availability and on how herbivory is modulated by diversity. However, it also delves into the multifaceted world of root systems, hyphal production, and fungal communities within mixed forest plantations, assessing their contributions to carbon sequestration and further extends to chlorophyll a fluorescence, emphasizing its role in shaping ecosystem dynamics (ED). Examining monocultures and mixtures composed of two species sheds light on the impacts of biodiversity on these processes.

Notably, this research offers insights into the adaptation strategies employed by tree species in response to diverse environmental conditions, particularly regarding herbivore interactions. The findings provide a mechanistic understanding of how tree diversity and species identities influence ecological processes and promote the conservation of mixed-species forest plantations.

This study is not confined to merely testing the effects of tree species mixing but endeavors to elucidate the underlying biological mechanisms, employing trait-based approaches. This approach seeks to generalize experimental findings and facilitate science-based recommendations for forest management and silviculture.

The study bridges the gap between different trophic levels, emphasizing interactions between producers (trees) and primary consumers (insects). It explores the complementarity and competition

dynamics between trees and insect herbivores while considering the overarching theme of drought's impact on ecosystems.

In conclusion, this work offers a multifaceted exploration of the intricate relationships between tree species diversity, water availability, and ecosystem functionality in Mediterranean ecosystems. The findings have significant implications for enhancing the ecological and economic benefits of mixed forest plantations while bolstering their resilience in the face of climate change.

These results are particularly pertinent to Mediterranean biomes and highlight the potential of mixed forest plantations as nature-based solutions to combat climate change and protect biodiversity.

Biodiversity; water stress; mixed forest plantations; ecosystem functionality; Mediterranean ecosystems; herbivore interactions

Declaration

I, the undersigned Tony Chahine, declare that this dissertation is the original report on my doctoral research at the University of Sassari, it has been written by myself and has not been submitted or presented, in whole or in part, for the award of any other academic degree or diploma elsewhere.

Tony Chahine

(2020-2023)

Acknowledgments

I would like to acknowledge everyone who played a role in my academic accomplishments. My deepest appreciation goes to my advisor, Dr. Simone Mereu, for their steady encouragement, insightful feedback, and invaluable expertise.

I wish to express profound gratitude to the University of Sassari (UNISS) for its foundational support and essential resources that have played a pivotal role in the successful completion of this Ph.D. journey. Additionally, I extend my special appreciation to the National Research Council (CNR) and the Euro-Mediterranean Center on Climate Change (CMCC) for their invaluable contributions, which significantly enhanced the outcome of this research endeavor.

A big thanks to all the people I have known and met along this path, who have contributed to this work.

Thank you all for your unwavering support.

General Index

List of Abbreviations

AIC	Akaike Information Criterion
ARPAS	Regional Agency for Environmental Protection
BA	Basal Area
BEF	Biodiversity and Ecosystem Functioning
CAMBIO	Climate Change Adaptation and Mitigation with Biodiverse Forest Plantations
CC	Climate Change
CE	Complementarity Effect
ChLF	Chlorophyll Fluorescence
CO2	Carbon Dioxide
CWM	Community Weighted Mean
ED	Ecosystem Dynamics
ES	Ecosystem Services
EU	European Union
FAO	Food and Agriculture Organization
FD	Functional Diversity
FDis	Functional Dispersion
Gsmax	Maximum stomatal Conductance
HSM	Hydraulic Safety Margins
INRA	National Institute of Agricultural Research
IPCC	Intergovernmental Panel on Climate Change
IPBES	Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services
IDENT	International Diversity Experiment Network with Trees
LA	Leaf Area
LAR	Leaf Area Removed
LLS	Leaf Life Span
LMA	Leaf Mass Area
LMM	Linear Mixed Model
MFC	Mix for Change
Mh	Maximum Height
NE	Net Diversity Effect
Nm	Nitrogen per mass
PCA	Principal Component Analysis
PLC50	Percentage Loss of Conductivity (50%)
Pnmax	Maximum Photosynthetic Rate at 25 °C
PSII	Photosystem II
SDW	Seed Dry Weight
SE	Selection Effect
SGH	Stress Gradient Hypothesis
SLA	Surface Leaf Area
SR	Species Richness
VOCs	Volatile Organic Compounds

WD   Wood Density
WP   Work Package

I. Chapter 1: Introduction

I.1 Fears surrounding the loss of biodiversity on a global scale

The earth is facing enormous environmental challenges, which are principally caused by unsustainable living standards for a vast growing share of the human population. Generally, these well-off people experience an urban life in a highly-globalized world and the total number of Earth's inhabitants is expanding, with realistic predictions counting as much as 9.7 billion people by mid-2050 (United nations, 2017).

The expansion of land area for intensive agriculture and urban development is globally omnipresent, but particularly so in the tropics (Lambin and Meyfroidt 2011). Also, nitrogen deposition affects abiotic conditions and the outcome of species interactions, so that this form of pollution is ranked among the largest drivers of environmental change (Millennium Ecosystem Assessment 2005a; Rockström et al. 2017). Without intending to be exhaustive, the Global redistribution of species, and notably the introduction of invasive alien species, is finally modifying many habitats (Early et al. 2016).

Changes in mean and extreme climatic conditions are affecting forest functioning worldwide (Frank et al., 2015). Moreover, the functioning of global biogeochemical cycles is further impacted by other drivers as land use change and pollutants: together these drivers are also causing a major loss in global biodiversity that many believe is the onset of the 6th mass extinction event.

Understanding and predicting these impacts is necessary for science-based decisions, but challenging because climate change interacts with other drivers of global change, such as rising atmospheric Carbon Dioxide (CO_2) (Cramer et al., 2001), atmospheric deposition (De Vries et al., 2014), land use change, pests, invasive species, management and legacy effects (Baudena et al., 2015). The science of climate change, coordinated by the Intergovernmental Panel on Climate Change (IPCC), and that of biodiversity, coordinated by Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services

(IPBES) have worked separately in the past decades and it is only recently that the two communities have recognized that these two major threats to our livelihoods are strongly interconnected and

represent one unique challenge: drivers of climate change and of biodiversity loss are basically the same but the interconnection is even stronger because biodiversity is an intrinsic component of ecosystems and affects ecosystem functions and services that could not occur otherwise.

The relatively preserved natural habitats are being lost at an unprecedented pace as expanding human population are converting these habitats to urban areas, roads, agricultural land and industrial zones (Anderson, 2002). Human pressures on the environment initially have spatial impacts which include deforestation, pollution of water bodies, land degradation and urbanization; secondly have temporal impacts such as climate change, ocean acidification with serious consequences on biodiversity as well as on human economies. (Costanza et al., 2014).

Excessive human development has eroded these resources with overexploitation of natural resources and destruction of natural habitats (Kareiva, Watts, McDonald, and Boucher, 2007).

Numerous regions (mainly in the tropics) have faced dramatic plant species losses due to the conversion of primary vegetation into intensive agricultural systems (Laurance, Sayer and Cassman 2014), very little changes in biodiversity are found in other regions, and some regions have seen a positive trend. Managed urban green areas, for instance, harbor several plant species (especially neophytes) thanks to accidental and deliberate species introductions and thanks to the human-mediated creation of environmental heterogeneity. A review study conducted by Vellend et al. (2017) conclude that the impacts of temporal biodiversity change drivers (e.g. habitat fragmentation, climate warming, nitrogen deposition) are context-dependent, therefore that biodiversity evolutions vary substantially at local scale and long-term predictions are far from certain (Figure 1).

This uncertainty also holds at regional scale, where colonization rates by non-native species are typically greater than the number species that have gone regionally extinct. At global scale, greater species extinction due to anthropogenic pressure are partially balanced by species speciation, although future biodiversity declines are likely due to increased habitat conversion in the tropics and due to extinction debt from current transitions.
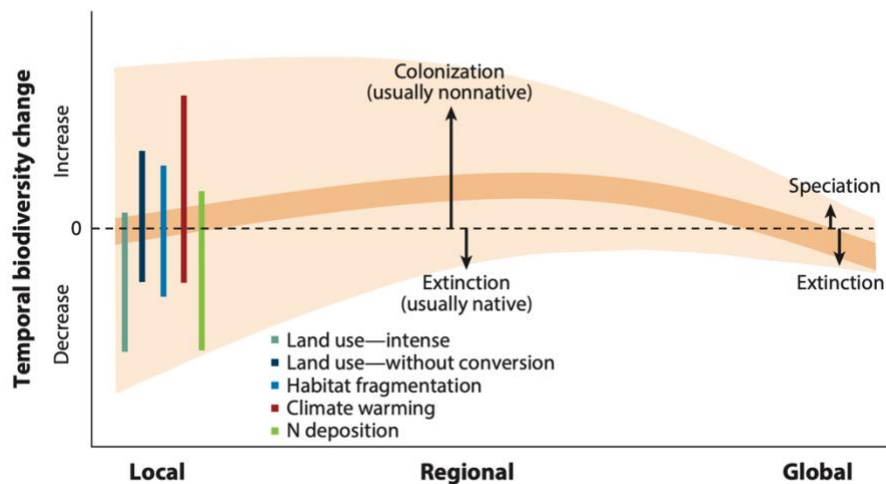
Figure 1 *Temporal trends of plant biodiversity change during the Anthropocene at different spatial scales. Locally, effects of major drivers of change are highly context-dependent (bars represent variation of possible outcomes). This induces an enormous variation in temporal trends between different localities (light zone), which level out to zero on average (dark zone).*

At regional scale, establishment of non-native species usually outranges extinction of native species and causes an average increase in plant biodiversity. Global biodiversity trends are a tradeoff between extinction and speciation rates, which have both increased owing to human activities. Global extinctions have the potential to, by far, May soon exceed speciation (Source: Vellend et al. 2017).

I.2 Mixed tree species plantation perspective

Forests have helped sustain humans for millennia; from the water we drink to the houses we reside in, forests provide us with a wealth of goods and services. Ecosystems react to climate change in complex ways, for example through stabilizing processes (Lloret et al., 2012) such as positive biotic interactions or local adaptation and phenotypic plasticity (Benito-Garzón et al., 2019), but also with destabilizing non-linear responses and feedbacks that could trigger tipping points (Camarero et al., 2015).

To support the crucial role of forests in maintaining key ecosystem services (ES), decision-makers must develop adaptive forest management strategies for the future changes.

To aid this process, it is therefore critically important to rapidly increase our ability to predict forest responses and vulnerability to climate change.

Forest landscape restoration, afforestation, and tree planting have recently gained significant international attention as key opportunities to mitigate climate change through carbon sequestration.

Yet, the ongoing increase in biotic and abiotic stress driven by climate change puts forests under threat. A growing body of evidence suggests that mixed forest plantations where several tree species and varieties are mixed, are more efficient in sequestrating carbon, while better coping with climate change-related stress. Conversely, monocultures still dominate the world's forest plantations.

Forest plantations that are diverse in genotypes, species, structure, and function, should be better able to adapt to changing environmental conditions than monocultures (Bauhus et al. 2010). This calls for the development of novel, more diversified forest plantations that can improve plantations' stability, productivity, and delivery of ecosystem services.

Promoting the large-scale expansion of climate change-resilient mixed forest plantations will require to foster the scientific base for mixed forest management to reduce and to identify and address in future forest policies the reasons for the apparent reluctance of landowners and stakeholders to adopt mixed plantations. The general among landowners and stakeholders and the aim is to identify which mixtures provide higher levels of biomass production and of other ecosystem services and how environmental conditions affect the relationship between tree diversity and forest functioning both in space and time.

Plantation forests are almost exclusively planted as monocultures., Despite this, several reviews published recently provide evidence, from both natural forests and plantations that biomass production and the delivery of other ecosystem services increase can progress with tree diversity (Nadrowski et al. 2010).

Furthermore, global change may increase disturbance frequencies and intensities in both natural forest and plantations (Woods et al. 2005), significantly affecting wood supply chains with severe economic consequences (Hanewinkel et al. 2012).

A new perspective of plantations under new objectives could have profound and rapid impacts on the social acceptance of forestry and it has been noted, that foresters currently resist establishing mixed plantations, in large parts because of the perception that mixing genotypes and species reduces yield and complicates forest management operations (Carnol et al. 2014).

It is imperative to define the overarching concept of ecosystem services. Ecosystem services are the countless benefits that ecosystems give upon both human societies and the environment.

These services can be categorized into four main types: provisioning services, which include the production of food, water, and raw materials; regulating services, encompassing climate regulation, disease control, and natural hazard mitigation; supporting services, such as nutrient cycling, soil formation, and habitat provision; and cultural services, embracing the aesthetic, recreational, and spiritual aspects of human well-being. The focus is elucidating on the elaborate connections between tree diversities, environmental conditions, and specific types of ecosystem services. The simulation models employed in this study serve as invaluable tools to systematically explore the dynamic interactions within ecosystems, predicting the outputs of ecosystem services across a spectrum of tree diversities and environmental conditions. By delving into the complex interplay of these factors, we aim to contribute to a nuanced understanding of how trees, with their lifespan and considerable size, play a pivotal role in shaping and sustaining the ecosystem services upon which we depend.

Given the long lifespan and size of trees, simulation models that predict ecosystem service output along a range of tree diversities and environmental conditions are an obvious approach. However, such models need parameterization, which is an enormous challenge given how poorly we understand biotic interactions among species. Parameters can be estimated based on experiments or observational studies, but both the types and ranges of tree diversities we seek to study are not always present. Observational studies are invaluable for providing a real-world Reference data (Baeten et al. 2013), but also have various drawbacks because tree species composition strongly depends on environmental factors or management. Experiments avoid these issues, but there are still relatively few experiments with replicated stands of mixed species (Scherer-Lorenzen 2014), and lots of these uses only a small number of tree species for commercial and timber production purposes.
Notably, highly interesting and relevant work has been accomplished with simulation tools (Morin et al. 2011).

I.3 Species interaction and drought resilience in forests

Increasing durations and frequencies of droughts under climate change endanger the sustainable functioning of forests worldwide (Haberstroh and Werner, 2022). There is an urgent need to better understand the processes enhancing tree resilience towards extreme climatic events and tree resilience must be assessed as the resistance to a drought event, as well as tree recovery to fully capture the impact of a drought (Sensu Ingrisch and Bahn, 2018; Gessler et al. 2020).

To achieve a higher resilience of forests and trees to droughts, one of the most prominent recommendations is the conversion of monospecific stands into mixed-species forests (Messier et al. 2021). In numerous cases, it has been demonstrated that such mixtures exhibit higher growth than the respective monocultures (Zhang et al. 2012), even under moderate drought stress (Lebourgeois et al. 2013; Ammer 2019). The main feature which drives this so-called diversity–productivity relationship is the complementarity of species resource use strategies, which include interspecific facilitation, competition release or resource partitioning (i.e. positive species interactions).

However, there is also evidence that competition effects (i.e. negative species interactions) can induce negative effects for some species in diverse forest ecosystems. Thus, tree diversity alone cannot be the only determining factor for beneficial species interactions (Grossiord, 2020). Instead, it has been stated that diversity in functional species traits (Bongers et al. 2021) and diversity in hydraulic strategies (Werner et al. 2021) play major roles for tree and ecosystem drought resilience. For example, positive effects on tree water-use efficiency were absent in a two-conifer mixture where both tree species exhibited similar rooting patterns (Grossiord et al. 2014a), while niche complementarity in water uptake was observed between shallow-rooted *Pinus sylvestris* L. and deep-rooted *Quercus petraea* (Matt.) Liebl (Bello et al. 2019).

In order to assess the impact of species interactions on tree resilience, resistance and recovery in response to drought, (Haberstroh and Werner, 2021) compared the water fluxes of a tree species in its monoculture to the water fluxes of the same species in a forest mixture during (resistance) and after (recovery) maximum drought stress (Figure 2), in this context, higher water fluxes of a species growing in a mixture compared to the same species in a monoculture during maximum drought stress would depict an increase in tree resistance to drought and point to the dominance of positive species interactions, such as facilitation. Conversely, reduced water fluxes of a species in mixture compared to its monoculture during maximum drought are defined as decreased resistance to drought, and indicate the dominance of negative species interactions, such as competition effects.
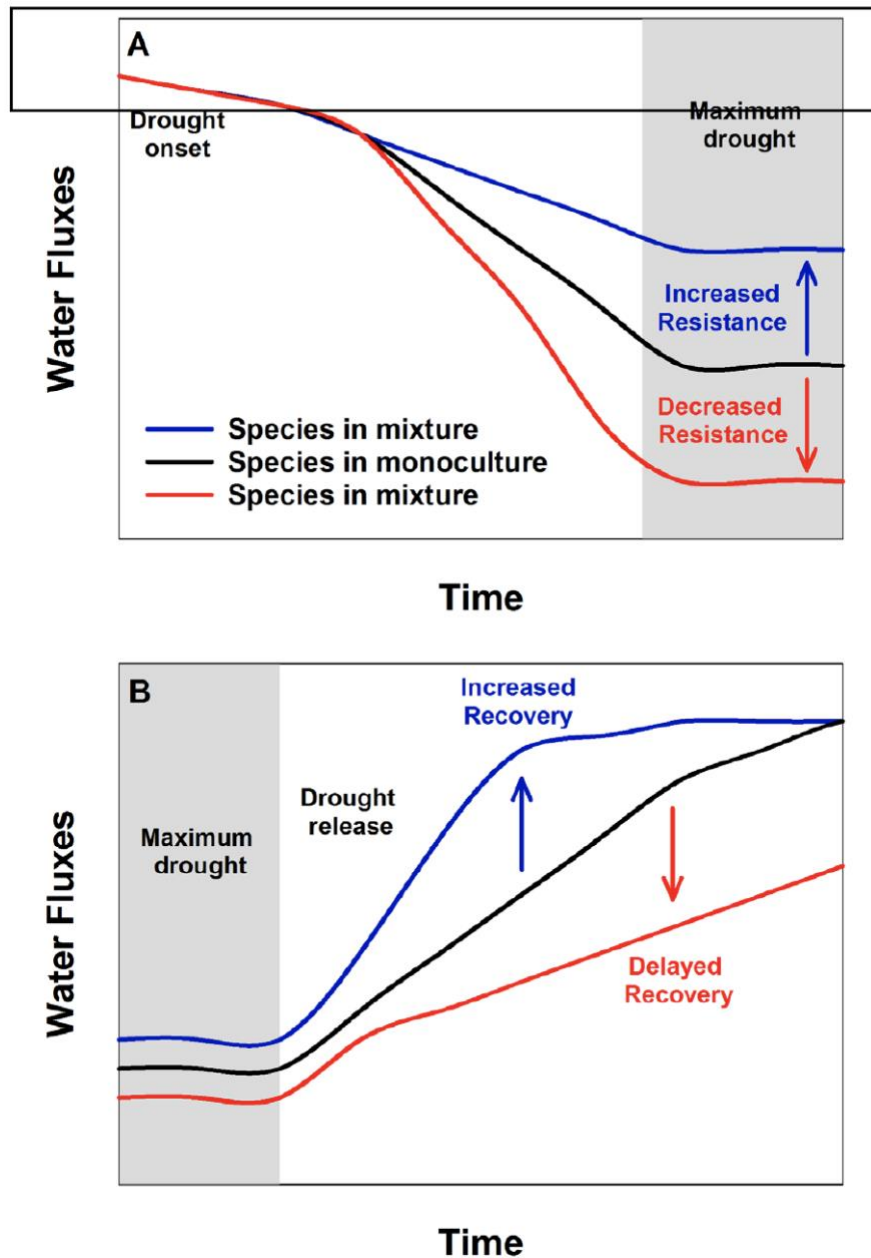
Figure 2 *Conceptual illustration of positive and negative impacts of species interactions on tree resistance (A) during maximum drought and on tree recovery (B) after maximum drought. Higher water fluxes of a species in a mixture compared to the species growing in a monoculture during/after maximum drought stress are defined as an increase in tree resistance/recovery to drought. Lower water fluxes of a species in a mixture compared to the species growing in a monoculture during/after maximum drought stress are defined as decrease in tree resistance/recovery to drought. The shaded area depicts the maximum drought stress. (source: Haberstroh and Werner, 2021).*

I.4 Influence of species interactions on tree resistance in response to increasing severity of drought

The analysis of drought responses and drought severity suggested three different response patterns for tree resistance towards drought, depending on drought severity (Mild, Moderate – Severe and Extreme).

Under mild water stress, beneficial species interactions increased tree species resistance towards drought. These positive effects persisted for some tree species at moderate to severe drought stress. Yet, for other tree species, this level of drought stress was adequate to iduce a shift from beneficial to negative species interaction effects. This adverse impact on tree resistance to drought finally became dominant under extreme water scarcity in most of the studies (Haberstroh and Werner, 2021).

Increased tree resistance toward drought under mild drought stress

Increased tree resistance towards drought observed under mild drought stress point towards beneficial species interactions and fit the concept of above- and belowground complementarity of species during times of enough water supply (Robles et al. 2020). Belowground, co-occurring species with different rooting patterns can partition soil water resources more efficiently (Bello et al. 2019), as different soil compartments are utilized to fuel transpiration (Grossiord, 2020). For example, *Fagus sylvatica* L. was able to access deeper soil water resources, when co-occurring with an increasing share of conifers (Grossiord et al. 2014b). Aboveground, trees with contrasting crown architecture, canopy stratification or shade tolerance (Pretzsch, 2014) elucidate an increased light-use efficiency and absorption of photosynthetically active radiation, which could also contribute to the observed patterns. For example, a mixture *of Fagus sylvatica* L. and *Picea abies* (L.) Karst. yielded an increased light interception and water-use efficiency because of a release in intraspecific competition (Pretzsch et al. 2012).

I.5 Increasing drought stress has adverse effects on tree resistance towards drought

Even under moderate to severe droughts, the same positive effects on tree resistance towards drought as under mild water stress can persist, especially the admixture of conifers with broadleaved species appears to be advantageous for tree transpiration resistance to drought (Grossiord et al. 2014b; Bello et al. 2019; Magh et al. 2019). However, there is evidence that under increasing drought stress, species interactions can shift from a positive to a negative interaction because of increasing niche overlap (Rodrı́guez-Robles et al. 2020) or competition effects Haberstroh et al. 2021). For example, in mixtures of *Fagus sylvatica* L. and *P. abies*, (Schume et al. 2004) reported *that F. sylvatica* was highly competitive belowground, forcing *P. abies* to grow more roots in the upper soil layers, potentially rendering shallow-rooted *P. abies* even more susceptible to drought (Goisser et al. 2016).

I.6 Negative species interactions in response to extreme water scarcity

The adverse patterns under moderate to severe drought already suggest that negative species interactions might prevail and become dominant under extreme water scarcity, many tree species suffer from the admixture of another species under extreme drought. This is highly relevant, as forests in Europe and elsewhere will experience an increase in extreme drought events (IPCC, 2021). These patterns may also be valid for other ecosystems. In a boreal ecosystem, the conifers *Pinus sylvestris* L. and *Picea abies* (L.) Karst strongly suffered from the admixture of broad-leaved *Betula pendula* Roth, resulting in reduced water-use efficiency for the conifer tree species (Grossiord et al. 2014a). During the extreme drought in 2003, both *Fagus sylvatica* L. and *Picea abies* (L.) Karst trees expressed lower net primary productivity in mixed stands compared to trees in their respective monocultures in a temperate forest (Pretzsch et al. 2012). These patterns demonstrate that even species that are complementary in their resource use (Grossiord et al. 2014c) experience increased competition under extreme drought, which can result in the dominance of one species under extreme water scarcity.

I.7 Biodiversity and ecosystem functioning (BEF)

There is solid evidence that biodiversity increases the stability of ecosystem processes in changing environments, Conversely the mechanisms that trigger this effect are still controversial and poorly understood. As the relationship between biodiversity and forest functioning is primarily explored in mature systems, facilitative interactions in the early forest stages are far less understood, notably in the context of restoration success. To meet these shortcomings, research started manipulating tree species composition and diversity at scales relevant to policy and management (Verheyen et al., 2015). In these so-called tree diversity experiments, contrasting results have yet been found about the effects of tree diversity on seedling performance. Negative effects are observed by Yang et al. (2013), neutral effects by Potvin and Gotelli (2008) and Yang et al. (2017), and species-dependent diversity effects by Van de Peer et al. (2016).

The interest in restoration pathways via positive nurse plant seedling interactions aligns with a growing research on the functional significance of biodiversity under climate change (Cardinale et al., 2012; Tilman et al., 2014). It was reported that the adoption of polyculture plantations, instead of conventional large-scale monocultures, provides long-term benefits for dealing with climatic uncertainties (Pawson et al., 2013). Initially, mixed forests benefit from greater spatial and temporal stability; a consequence of asynchrony in species responses to environmental fluctuations (Jactel et al., 2017). Secondly, ecosystem functioning (e.g. productivity, litter decomposition) is favored in mixed stands through resource complementarity (Madrigal-gonzález et al., 2016) or through the regulation by higher (Castagneyrol et al., 2014) or lower trophic levels (Laforest-lapointe et al., 2017).

I.8 Ecosystem stability

The relationship between biodiversity and stability has been the subject of a long-standing debate in ecology (Pimm 1984; McCann 2000; Loreau et al. 2002; Ives and Carpenter 2007). Current experimental studies declare that diversity may stabilize aggregate ecosystem or community properties while simultaneously destabilizing individual species abundances (Tilman 1996; Tilman et al. 2006; Hector et al. 2010).

The new concept of biodiversity-stability tends to trigger changes in all ecosystem processes as forests (Thompson et al. 2009; Jucker et al. 2014), grasslands (Tilman et al. 2006; Hector et al. 2010) and aquatic systems (Boyer et al. 2009), these examples confirm that diverse ecosystems benefit from higher temporal and spatial stability with environmental stress or with ecological disturbances.

Species interactions help buffer the impacts of environmental stress and disturbance to greater diversity and help maintain the long-term functioning of the ecosystem. For example, facilitative interactions that improve water availability or water uptake efficiency reduce the occurrence of severe water stress, thereby improving ecosystem stability. Among these facilitative interactions is hydraulic lift whereby deeper rooting tree species consume water in deep soil layers and redistribute this water to superficial soil layers in mixed forests (Jactel et al. 2017).

Besides resistance to drought, wind damage may be lower in mixed forests, among various other reasons because wind impacts are distributed over multiple forest layers so that wind loading on the tallest trees is relaxed. What is more, the pest populations in homogeneous communities of host plants can rapidly build-up and can dramatically reduce ecosystem functioning. Therefore, the associational resistance is essential to explain biotic stability.

Latest findings verify that the biodiversity-stability concept offers essential management prospects for improving ecosystem resistance and resistance and recuperation from natural disasters in the light of global change.

I.9 Functional diversity (FD)

The relations between plant diversity and ecosystem functioning remain highly debatable and as defined by Petchey and Gaston (2006) the functional diversity is a component of biodiversity that generally concerns the range of things that organisms do in communities and ecosystems.

Functional diversity generally involves understanding communities and ecosystems based on what organisms do, rather than on their evolutionary history. This is a very general definition for functional diversity and an enormous amount of ecological research is relevant.

For example, if "what organisms do' is interpreted as the organisms' phenotype (i.e. a phenotypic trait) then functional diversity equates with phenotypic diversity and most of the ecological research has touched on this subject. While such generality is acceptable, recent research about the potential consequences of biodiversity for ecosystem processes (Tilman 1999; Chapin et al. 2000; Grime 2001; Loreau et al. 2001; Hooper et al. 2005) has led to a more specific definition: "the value and range of those species and organismal traits that influence ecosystem functioning' (Tilman, 2001). A consequence of this definition, one that pervades this review, is that measuring functional diversity is about measuring functional trait diversity, where functional traits are components of an organism's phenotype that influence ecosystem level processes.

A wide range of key ecological questions can be addressed in terms of functional diversity. There are purely descriptive questions concerning, for example, the nature of latitudinal gradients in functional diversity and whether these differ from what one expects by chance (e.g. Stevens et al. 2003). There are questions about the evolutionary and ecological determinants of functional diversity (e.g. Weiher et al. 1998). Functional diversity can also address questions about determination of ecosystem level processes (Chapin et al. 2000; Díaz and Cabido 2001) and is a concept that links species and ecosystems through mechanisms such as resource use complementarity and facilitation. Measuring functional diversity requires, ideally, each of the

following:

1. Appropriate functional information (traits) about organisms to be included in the measure, and irrelevant information to be excluded (what functional traits should be included?).

2. Traits to be weighted according to their relative functional importance (Petchey and Gaston 2002a; Roscher et al. 2004).

3. The statistical measure of trait diversity to have desirable mathematical characteristics (Mason et al. 2003; Botta-Dukát 2005; Ricotta 2005). For example, discontinuous vs. continuous measures of diversity.

4. The measure to be able to explain and predict variation in ecosystem level processes.

I.10 Species diversity and composition as essentials of carbon sequestration

Carbon sequestering both above and belowground carbon has been recognized in the context of the Clean Development Mechanism of the Kyoto protocol (Thomas et al. 2010). Using reforestation and afforestation to create carbon sinks while taking biodiversity concerns into account provides a decent example of the potential contributions of the new concept of experimental tree plantations. However, the choice of genotype and species, each with different carbon sequestration time profiles, and the positive or negative effects of mixtures for maximizing carbon sequestration rates in forest plantations at different sites across the globe are still open to debate.

According to the Food and Agriculture Organization's (FAO) Global Planted Forest Assessment Database Global Planted Forest Assessment database (FAO, 2006), the total number of species used in plantations ranges from 4 in Finland to 20 in China, France, India, and Ukraine. The recent studies experimental plantations suggest that the carbon sequestration rates of tree species that are rarely planted in forestry may be higher than for species that are traditionally planted for wood production and the observations confirm the presence of species identity effects, which underlines the importance of increasing the number of species used in forest plantation projects.

Tree species diversity is known to promote key forest ecosystem functions, including primary production (Paquette and Messier 2011; Liang et al. 2016), stability of wood production (Jucker et al. 2014), resistance to biotic and abiotic disturbances (Pretzsch et al. 2013b; Jactel et al. 2017) and nutrient cycling (Richards et al. 2010; Handa et al. 2014), amongst others. Despite evidence that diverse forests can support higher levels of ecosystem functioning than species-poor ones (Gamfeldt et al. 2013), the importance of tree diversity as a driver of ecosystem functioning is also known to

vary considerably amongst forest types, geographic regions, and in relation to climatic conditions (Paquette and Messier 2011; Ratcliffe et al. 2016). This context dependency of diversity effects is seen as an obstacle to scaling up and generalizing biodiversity experiments, because the importance of environmental conditions in shaping biodiversity and ecosystem functioning (BEF) relationships is poorly understood, particularly for ecosystem functions other than biomass production (Cardinale et al. 2000; Allan et al. 2015).

I.11 Does the mixture optimize the control of insect pests in tree plantations?

Climate change with increasing temperatures and more frequent drought events is expected to aggravate forest pest damage through increased pest proliferation or reduced plant defense (Jactel et al. 2012) further more plant diversity has often been reported to decrease insect herbivory in plants. Of the numerous mechanisms that have been proposed to explain this phenomenon, how plant diversity influences plant defenses via effects on growth has received little attention. In addition, plant diversity effects may be contingent on abiotic conditions such as resources and water availability (Galmán et al. 2022).

With known abiotic disturbances such as storms or fires, the biotic damage can dramatically alter the functioning of forest ecosystems and reduce their productivity. For instance, every year, on average 15–20 % of the trees in European forests are affected by pest and pathogen damage, resulting in increased tree mortality or reduced tree growth. In response to the need for in-depth knowledge of the functioning of mixed plantations and the services they provide, it's critical to better understand the significance of forest diversity for the forest's resistance to pest insects and its resilience to their outbreaks.

The direction and strength of the interaction does not only occur at a physiological and neighboring level. Other interaction emerges when larger spatial scales or longer time periods are considered. at these scales for example multitrophic interactions may emerge as in the case of herbivore dynamics. Furthermore, the impact of herbivore species may be lower in more diverse plant communities due to mechanisms of associational resistance. According to the "resource concentration hypothesis" the amount and accessibility of host plants is reduced in diverse plant communities, thus limiting the

exploitation of resources by consumers. In addition, the "natural enemy hypothesis" suggests that richer plant assemblages provide natural enemies with more complementary resources and habitats, thus promoting top down regulation of herbivores (Guyot et al., 2015) and reducing the risk of pest damage in general (Jactel et al., 2002).

I.12 Reducing risk of pest damage

the most difficult aspect of mixtures to study is using species mixtures is a reduction of insect or disease damage as damage generally occurs episodically and is affected by vegetation composition and structure at the landscape levels. For the most part, studies that are designed to address questions of competition, nutrition, and productivity on small plots cannot provide information on insect and disease damage. Much of the research that does focus on pest effects compares monocultures to complex natural forests (Jactel et al., 2002). Many potential managers of mixed-species plantations would be interested in whether only 2, 3, 4 or 6 species in mixture reduce risk compared to monocultures, and there is very little information about these low-diversity mixtures. The potential risk of monocultures is that the invasion of a pest would affect all or most of the trees because of the uniform genetic composition. Recent assessments on the vulnerability of plantations (Powers, 1999; Gadgil and Bain, 1999) noted that most plantations (which are nearly all monocultures) have low incidences of insect and disease problems, but stressed the importance of two factors: (I) monocultures must be managed well, especially in terms of control of stand density; periodic thinning must preserve individual tree vigor high to reduce the impacts of most pest species; (II) many monocultures are not native to the region where they have been planted, and their natural pest species have not been introduced with the trees. Despite these factors, considerable risk is still involved, because many plantations experience periods of poor management, and pest species (along with most other types of organisms) are increasingly being moved around the globe. Two mechanisms by which mixtures may reduce risk of pest damage are:

1. Mixtures may dilute the host concentration for a pest organism and thereby impair the ability of the pest to find the host (Kelty, 2006)
2. Mixtures may provide more diverse habitats that tend to support higher populations of natural enemies of the pest species (Watt, 1992)

There is still little work on many aspects of the role of species mixtures in plantation such as the prevalence and mechanisms of beneficial below ground interactions; the effects of site factors and spacing on species interactions; and the prevalence of beneficial interactions across a broad range of tree species. Experiments on the mechanisms of interactions among species are so expensive that most are carried out on very small plots, nearly all are on one site type and at one spacing level, and only a small number of tree species have been included. Most studies have used replacement series designs, which focus on variations of species proportions at a constant overall spacing (Sackville Hamilton, 1994; Kelty and Cameron, 1995). Some also include additive series to incorporate variation in spacing, and some include both synchronous and delayed planting, where one species has a very rapid juvenile growth rate. However, it is unlikely that funding will be available to expand many replicated replacement/additive experiments to include all these essential factors. A variety of research approaches is needed, including species trials, continued studies of interaction mechanisms, and operational trials (Kelty, 2006).

I.12.1 Species trials

The most direct way to include more species is to expand upon the standard species trial, in which small monoculture blocks of trees are planted. This can be supplemented with a spatially randomized mixture of all the species in the monoculture blocks (e.g., Piotto et al., 2004; Erskine et al., 2005). These provide initial information on competitive interactions among species. It may be more informative to establish blocks of all possible two-species mixtures, but that would increase the land area and number of trees. Trees located on edges of adjacent monoculture blocks can be useful in examining interactions (Rothe and Binkley, 2001). Thus, small monoculture blocks in species trials could be arranged to create edges among all species involved in the trial.

I.12.2 Studies of species interactions

A combination of replacement and additive series plots is an important method to analyze interaction effects on stand development and productivity, but enough replication at the plot level quickly adds up to large land areas and high costs. A more efficient method is individual-tree analysis, which focuses on the effect of neighborhood tree variables (species, size, distance) on the growth of a subject tree. This kind of analysis can be done with any replacement or additive series experiment (Vanclay, 2006a), but plots can be designed to take advantage of neighborhood structures (Kelty and Cameron, 1995).

There are also innovative plot designs that are designed to be so efficient in land area that they can be used as species trials, and yet still allow quantitative analysis of species interactions at the individual tree-level. These have been described for two species (Nester, 1994), three species (Goelz, 2001) and four species (Vanclay, 2006b).

I.12.3 Operational trials

It is necessary to have a better connection of using research results to design operational scale trials with industry and with family/community owners of small forest tracts. This approach offers the greatest likelihood of testing a wider range of species combinations across various sites statistically described by Rothe and Binkley (2001) to increase the sample size for analysis of mixture results. Much research on monoculture plantations is conducted in operational plantings, which gives a great advantage in providing low cost replicates of research treatments. In addition, the adoption of mixed-species plantation silviculture will require forest managers to solve operation problems that are not encountered when planting small experimental plots, and any progress in this area will represent a major step in moving mixed-species plantations toward standard use in forest management.

I.13 Tree diversity experiments

There are currently at least 25 tree diversity experiments exploring the functional consequences of tree species mixing. Similar as in the conventional model systems, they encompass different species compositions in a replicated and semi-randomized design, with the inclusion of all monocultures as well as 2, 3, 4 or 6 species in mixture. Tree diversity experiments cover a total area of 821 ha in different climates (Figure 3). They have been developed through independent initiatives, but they participate in a global platform network (TreeDivNet) where synthesis work is assisted and field expertise is shared (Verheyen et al. 2015). Close affinity with common silvicultural practices is generally objected, but still, experiments differ remarkably in terms of diversity index manipulated (species richness SR, functional diversity, phylogenetic diversity, genetic diversity and evenness), upper-diversity level of species richness (from 2 to 18 species), type of tree species mixing (either patch planting or individual-based planting), spatial scale (plots sizes ranging from 0.24 to 12 000 m^2), planting density (between 40 cm and 300 cm, but usually constant within experiments), site heterogeneity (e.g. from flat sites to hilly slopes) and more (Verheyen et al. 2015). Only 13 experiments were established before 2010.

Figure 3. *Global distribution of tree diversity network experiments contributing to TreeDIvNet (www.treedivnet.ugent.be). TreeDivNet offers a science-based research platform for discovering the relationships between tree species diversity and ecosystem functioning through experimental approaches (Verheyen et al. 2015). The 25 experiments cover dominant ecoregions, including Boreal, Temperate, Mediterranean, and Sub-tropics. Because of their independent establishment, plantations use various design alternatives and their age ranges between 1 to 19 years old. (Source: www.treedivnet.ugent.be).*

I.14 Scope of the study

Several decades of intensive ecological research and studies generated profound and solid knowledge on how biodiversity controls ecological processes that are fundamental to the functioning of ecosystems. However, almost all manipulative biodiversity experiments carried out so far have used fast-growing and small-scale model systems in laboratory environments. Also, the experimental work in the field was mainly limited to grassland systems and on early stage plantation.

This thesis was consequently encouraged by a vast call from science, policy and management to test the validity and relevance of biodiversity and ecosystem functioning (BEF) relationships and underpinning mechanisms for one of the most important and most complex real-world ecosystems which are the forests.

The study underscores the importance of biodiversity in adaptation strategies to global change in Mediterranean ecosystems and emphasizes the need for greater efforts to protect and promote biodiversity conservation.

This thesis addresses a complex investigation at the intersection of biodiversity, water stress, mixed forest plantations, and their effects on ecosystem functionality. The central focus of this study is the profound influence of biodiversity on controlling fundamental ecological processes vital to ecosystem function and stability. By inspecting diverse biodiversity profiles, this research seeks to unveil their impacts on the overall health of ecosystems. Furthermore, it aims to assess how alterations in biodiversity affect the resilience and stability of ecosystems.

The term "fundamental ecological processes" encompasses a range of interconnected mechanisms that drive the dynamics within ecosystems. These include, but are not limited to:

i-   Nutrient Cycling: Biodiversity plays a key role in regulating the cycling of essential nutrients within ecosystems. Different species contribute unique biochemical pathways, influencing the availability and distribution of nutrients, which is vital for the growth and development of organisms within the ecosystem.

ii- Biomass accumulation: The diverse array of plant species in mixed forest plantations contributes to enhanced Biomass accumulation. Through photosynthesis and subsequent storage in biomass and soils, biodiversity directly impacts the global carbon cycle, influencing climate regulation and mitigating the effects of climate change.

19

iii-   Water Regulation: Biodiversity can influence water availability and distribution within ecosystems. Different plant species have distinct water-use efficiencies and root architectures, affecting the retention and movement of water through soil, which in turn influences local hydrological cycles.

iv-   Species Interactions: Biodiversity is intricately linked to various ecological interactions, such as predation, competition, and mutualism. These interactions contribute to the resilience and stability of ecosystems by regulating population dynamics and maintaining biodiversity.

By examining diverse biodiversity profiles, this research aims to unveil the specific impacts of different plant species on these fundamental ecological processes. For example, the composition of a mixed forest plantation influences nutrient availability in the soil, alters carbon cycling dynamics, modulates water regulation patterns, and shapes the intricate web of species interactions within the ecosystem.

Another critical dimension of this study explores the Water Stress Gradient Hypothesis, delving into its validity and practical implications. Analysis will be conducted to understand how ecosystems respond to varying levels of water stress, investigating changes in primary productivity, community composition, and the adaptive strategies deployed by organisms in response to water availability fluctuations. This section will also consider the long-term sustainability and adaptability of ecosystems in the context of changing climate patterns and water resource availability.

Within mixed forest plantations, this research will inspect the complex network of root systems, hyphal production, and fungal communities. The quantification of their contributions to carbon sequestration will be a primary focus. Additionally, the study will analyze how diversity gradients within mixed forest plantations influence their exposure and vulnerability to drought, encompassing complex interactions among tree species and soil microorganisms, and the ensuing impacts on carbon storage and ecosystem stability.

Furthermore, the research will involve a specific examination of chlorophyll a fluorescence, concentrating on monocultures and mixtures composed of two species. This investigation will further illuminate the role of biodiversity in shaping ecosystem dynamics.

Additionally, the thesis aims to assess the influence of tree species diversity and water availability on growth and herbivore defense within the *Quercus ilex* L. .species. By examining these facets, the study intends to provide insights into the adaptive strategies employed by tree species under diverse environmental conditions and their potential implications for herbivore interactions.

In this PhD research, the overarching objective is to deepen our understanding of how ecological processes are influenced by tree species mixing. The primary focus is on enhancing our mechanistic understanding of the roles played by tree diversity and species identities within forest ecosystems. The research endeavors to provide science-based information, particularly for Mediterranean biomes, with the explicit goal of promoting mixed-species forest plantations as nature-based solutions to combat the causes and consequences of climate change. This involves not only assessing the impacts of tree species mixing on ecological processes but also striving to elucidate the underlying biological mechanisms. The work aims to move beyond a mere testing of ecological processes by employing trait-based approaches to detect and understand these mechanisms. The ultimate objective is to facilitate the generalization of experimental findings, with specific emphasis on informing forest management and silviculture practices. A significant aspect of this thesis centers on the interaction between two trophic levels—producers in a tree-centric framework and primary consumers as herbivores (insects). This approach involves targeting complementarity and competitive interactions between trees and insect herbivores for specific species, and the research investigates the effects of drought on the entire ecosystem, a theme that permeates the entirety of the study.

Stressing on forest management and silviculture advice, it is not enough to test how ecological processes are affected by tree species mixing. This study attempted elucidating underlying biological mechanisms, among others via trait-based approaches. Detecting and understanding biological mechanisms will help generalizing the experimental findings.

One part of this thesis focused on the interaction between two trophic levels which are producers as a tree-centric framework and primary consumers as herbivores (insects), which means that we targeted the complementarity and competitive interactions between the trees and insect herbivores for certain species and the research investigates the effects of drought on the ecosystem, a theme that permeates the entire study.

I.15 PhD outline
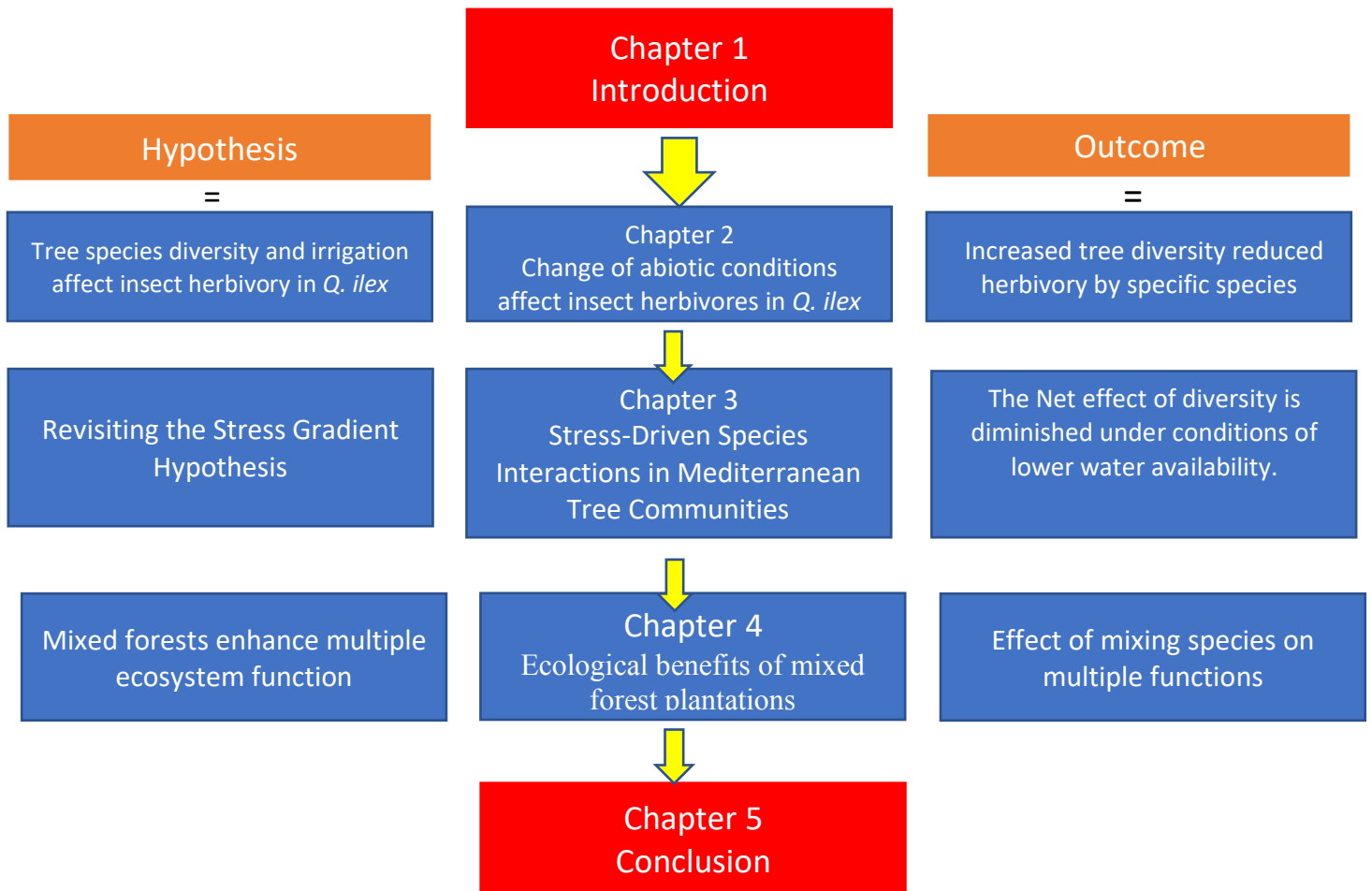
Figure 4. *Thesis outline. Each chapter in the left column describes an ecological concept studied, while the right column suggests the possible significance of the concept for plantation management and techniques.*

Chapter 1 of the PhD thesis delivers an introduction to the potential of biodiversity for adaptation strategies to global change in Mediterranean ecosystems. It outlines the research questions and objectives, focusing on identifying key ecosystem services provided by biodiversity in Mediterranean regions and some other examples and trials around the world and exploring how these services can be used to mitigate the impacts of global change specifically in both sites IDENT-MACOMER (Italy) and ORPHEE (France).

Chapter 2 investigates the influence of tree species diversity and water availability on growth and herbivore defense in *Quercus ilex* L. Findings indicate that tree species composition and water availability interact to shape defenses in *Q. ilex* leaves. Interestingly, water availability did not significantly impact on defenses, but the presence of pines in the mix led to considerably greater insect damage on *Q. ilex*.

Chapter 3 explores the Stress Gradient Hypothesis (SGH) in Mediterranean tree communities. It is found that biodiversity's impact on ecosystem functioning is influenced by species richness and functional diversity, with irrigation treatments playing a significant role. Contrary to the Stress Gradient Hypothesis, higher water availability enhanced biodiversity's effects.

Chapter 4 addresses the importance of forests and the limitations of monoculture forestry practices. It highlights the shift towards mixed forest plantations as a sustainable alternative, emphasizing their potential for carbon sequestration, improved soil quality, enhanced ecosystem services, and increased biodiversity. The synthesis of three recent studies offers a comprehensive overview of the ecological benefits of mixed forest plantations, with implications for ecological restoration and climate change mitigation.

Chapter 5 provides a conclusion of the implications and recommendations for future research and emphasizes the need for greater efforts to protect and promote biodiversity conservation in the Mediterranean ecosystems. Overall, the PhD thesis highlights the importance of biodiversity in global change adaptation strategies and new mitigation strategies in Mediterranean ecosystems and offers valuable information for policy makers, practitioners and researchers.

References

Allan, E., Manning, P., Alt, F., Binkenstein, J., Blaser, S., and Blüthgen, N. (2015). Land use intensification alters ecosystem multifunctionality via loss of biodiversity and changes to functional composition. Ecol. Lett., 18, 834–843.

Ammer C. (2019). Diversity and forest productivity in a changing climate. New Phytologist, 221, 50–66.

Anderson, C. L. (2002). Identifying important plant areas: A site selection manual for europe, and a basis for developing guidelines for other regions of the world. Plantlife International.

Baeten, L., K. Verheyen, C. Wirth, H. Bruelheide, F. Bussotti, L. Fine´r, B. Jaroszewicz, and F. Selvi (2013). A novel comparative research platform designed to determine the functional significance of tree species diversity in European forests. Perspectives in Plant Ecology, Evolution and Systematics 15: 281–291.

Bauhus, J., P. van der Meer, and M. Kanninen. (2010). Ecosystem goods and services from plantation forests. London: Earthscan.

Baudena, M., Sánchez, A., Georg, C.-P., Ruiz-Benito, P., Rodríguez, MÁ, Zavala, M.A., and Rietkerk, M. (2015). Revealing patterns of local species richness along environmental gradients with a novel network tool. Scientific Reports 5, 11561.

Bello J., Hasselquist N.J., Vallet P., Kahmen A., Perot T., and Korboulewsky N. (2019). Complementary water uptake depth of Quercus petraea and Pinus sylvestris in mixed stands during an extreme drought. Plant and Soil, 437, 93–115.

Benito-Garzón, M., Robson, T.M., Hampe, and A. (2019). TraitSDMs: species distribution models that account for local adaptation and phenotypic plasticity. New Phytologist, 222, 1757–1765.

Bongers F.J., Schmid B., Bruelheide H., Bongers F., Li S., von Oheimb G., Li Y., Cheng A., Ma K., and Liu X. (2021). Functional diversity effects on productivity increase with age in a forest biodiversity experiment. Nature: Ecology and Evolution, 5, 1594–1603.

Botta-Duka´t, Z. (2005). Rao's quadratic entropy as a measure of functional diversity based on multiple traits. J. Veg. Sci., 16, 533– 540.

Boyer, K.E., Kertesz, J.S. and Bruno, J.F. (2009) Biodiversity effects on productivity and stability of marine macroalgal communities: the role of environmental context. Oikos, 118, 1062-1072.

Camarero, J.J., Gazol, A., Sangüesa-Barreda, G., Oliva, J., and Vicente-Serrano, S.M. (2015). To die or not to die: early warnings of tree dieback in response to a severe drought. Journal of Ecology 103, 44–57.

Cardinale, B.J., Nelson, K. and Palmer, M.A. (2000). Linking species diversity to the functioning of ecosystems: on the importance of environmental context. Oikos, 91, 175–183.

Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., Narwani, A., Mace, G.M., Tilman, D., A.Wardle, D., Kinzig, A.P., Daily, G.C., Loreau, M., Grace, J.B., Larigauderie, A., Srivastava, D.S., and Naeem, S. (2012). Corrigendum: biodiversity loss and its impact on humanity. Nature 489, 59–67.

Carnol, M., L. Baeten, E. Branquart, J.C. Gre´goire, A. Heughebaert, B. Muys, Q. Ponette, and K. Verheyen. (2014). Ecosystem services of mixed species forest stands and monocultures: Comparing practitioners' and scientists' perceptions with formal scientific knowledge. Forestry 87: 639–653.

Castagneyrol, B., Jactel, H., Vacher, C., Brockerhoff, E. G., and Koricheva, J. (2014). Effects of plant phylogenetic diversity on herbivory depend on herbivore specialization. Journal of Applied Ecology, 51(1), 134-141.

Chapin, F.S., Zavelata, E.S., Eviner, V.T., Naylor, R.L., Vitousek, P.M., and Reynolds, H.L. (2000). Consequences of changing biodiversity. Nature, 405, 234–242.

Costanza, R., de Groot, R., Sutton, P., van der Ploeg, S., Anderson, S. J., and Kubiszewski, I. (2014). Changes in the global value of ecosystem services. Global Environmental Change, 26, 152–158.

Tony Chahine, The potential of Biodiversity for Adaptation Strategies to Global Change in Mediterranean Ecosystems. PhD thesis in Agrometeorology and Ecophysiology of Agricultural and Forest Systems, University of Sassari.

Cramer, W., Bondeau, A., and Woodward, F.I. (2001). Global response of terrestrial ecosystem structure and function to CO2 and climate change: results from six dynamic global vegetation models. Global Change Biology 7, 357–373.

De Vries, W., Dobbertin, M.H., Solberg, S., Van Dobben, H.F., and Schaub, M. (2014). Impacts of acid deposition, ozone exposure and weather conditions on forest ecosystems in Europe: an overview. Plant and Soil 380, 1–45.

Diaz, S. and Cabido, and M. (2001). Vive la diffe´rence: plant functional diversity matters to ecosystem processes. Trends Ecol. Evol., 16, 646–655.

Early, R., Bradiey, b.A, Oukes,. S, lawler, J.d, Olden, J. D. and Blumenthal, D.M. (2016) Global threats from invasive alien species in the twenty-first century and national response capacities. Nature Communications, 7, 12485

Erskine, P.D., Lamb, D., and Borschmann, G. (2005). Growth performance and management of a mixed rainforest tree plantation. New For. 29, 117–134.

FAO. (2006). Global forest resources assessment 2005, progress towards sustainable forest management. FAO Forestry Paper 147, Food and Agriculture Organization of the United Nations, Rome, Italy

Frank, D.A., Reichstein, M., and Bahn, M. (2015). Effects of climate extremes on the terrestrial carbon cycle: concepts, processes and potential future impacts. Global Change Biology 21, 2861–2880.

Gadgil, P.D., and Bain, J. (1999). Vulnerability of planted forests to biotic and abiotic disturbances. New For. 17, 227–238.

Galmán, A., Carla Vázquez-González, Gregory Röder and Castagneyrol B. (2022). Interactive effects of tree species composition and water availability on growth and direct and indirect defences in *Quercus ilex* L. Oikos 2022: e09125. doi: 10.1111/oik.09125.

Tony Chahine, The potential of Biodiversity for Adaptation Strategies to Global Change in Mediterranean Ecosystems. PhD thesis in Agrometeorology and Ecophysiology of Agricultural and Forest Systems, University of Sassari.

Gamfeldt, L., Snäll, T., Bagchi, R., Jonsson, M., Gustafsson, L., and Kjellander, P. (2013). Higher levels of multiple ecosystem services are found in forests with more tree species. Nat. Commun., 4, 1–8.

Gessler A., Bottero A., Marshall J., and Arend M. (2020). The way back: recovery of trees from drought and its implication for acclimation. New Phytologist, 228, 1704–1709.

Goisser M., Geppert U., R¨otzer T., Paya A., Huber A., Kerner R., Bauerle T., Pretzsch H., Pritsch K., H¨aberle K.H., Matyssek R., and Grams T.E.E. (2016). Does belowground interaction with Fagus sylvatica increase drought susceptibility of photosynthesis and stem growth in Picea abies? Forest Ecology and Management, 375, 268–278.

Grime, J.P. (2001). Plant Strategies, Vegetation Processes, and Ecosystem Properties. John Wiley and Sons, Inc., New York, NY.

Grossiord C. (2020). Having the right neighbors: how trees species diversity modulates drought impacts on forests. New Phytologist, 228, 42–49.

Grossiord C., Granier A., Gessler A., Jucker T., and Bonal D. (2014a). Does drought influence the relationship between biodiversity and ecosystem functioning in boreal forests? Ecosystems, 17, 394–404.

Grossiord, C., Granier, A., Ratcliffe, S., Bouriaud, O., Bruelheide, H., and Chećko, E. (2014b). Tree diversity does not always improve resistance of forest ecosystems to drought. Proc. Natl. Acad. Sci. U. S. A., 111, 14812–14815.

Grossiord C., Gessler A., Granier A., Pollastrini M., Bussotti F., and Bonal D. (2014c). Interspecific competition influences the response of oak transpiration to increasing drought stress in a mixed Mediterranean forest. Forest Ecology and Management, 318, 54–61.

Goelz, J.C.G. (2001). Systematic experimental designs for mixed-species plantings. Native Plants J. 2, 90–96.

Guyot V, Castagneyrol B, Vialatte A, Deconchat M, Selvi F, and Bussotti F. (2015) Tree Diversity Limits the Impact of an Invasive Forest Pest. PLoS ONE 10(9): e0136469. doi:10.1371 /journal.pone. 0136469.

Haberstroh, S., Caldeira, M. C., Lobo-do-Vale, R., Martins, J. I., Moemken, J., Pinto, J. G., & Werner, C. (2021). Nonlinear plant–plant interactions modulate impact of extreme drought and recovery on a Mediterranean ecosystem. New Phytologist, 231(5), 1784-1797

Haberstroh, S., and Werner, C. (2022). The role of species interactions for forest resilience to drought. Plant Biology ISSN 1435-8603.

Handa, I.T., Aerts, R., Berendse, F., Berg, M.P., Bruder, and Butenschoen, A. (2014). Consequences of biodiversity loss for litter decomposition across biomes. Nature, 509, 218–21.

Hanewinkel, M., D.A. Cullmann, M.J. Schelhaas, G.J. Nabuurs, and N.E. Zimmermann. (2012). Climate change may cause severe loss in the economic value of European forest land. Nature Climate Change 3: 203–207.

Hector, A., Hautier, Y., Saner, P., Wacker, L., Bagchi, R., and Joshi, J. (2010). General stabilizing effects of plant diversity on grassland productivity through population asynchrony and overyielding. Ecology, 91, 2213–2220.

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., and Lavorel, S. (2005). Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecol. Monogr., 75, 3–35.

Ingrisch S., and Bahn M. (2018) Towards a comparable quantification of resilience. Trends in Ecology and Evolution, 33, 251–259.

IPCC. (2021) Summary for policymakers. In: Masson- Delmotte V., Zhai P., Pirani A., Connors S. L., P´ean C., Berger S., Caud N., Chen Y., Goldfarb L., Gomis.

Ives, A.R. and Carpenter, S.R. (2007). Stability and diversity of ecosystems. Science, 317, 58–62.

Jactel, H., Goulard, M., Menassieu, P., and Goujon, G. (2002). Habitat diversity in forest plantations reduces infestations of the pine stem borer Dioryctria sylvestrella. J. Appl. Ecol. 39, 618–628.

Jactel, H., J. Petit, M.L. Desprez-Lousteau, S. Delzon, D. Piou, A. Battisti, and J. Koricheva. (2012). Drought effects on damage by forest insects and pathogens: A meta-analysis. Global Change Biology 18: 267–276.

Jactel, H., Bauhus, J., Boberg, J., Bonal, D., Castagneyrol, B., Gardiner, B., Gonzalezolabarria, J.R., Koricheva, J., Meurisse, N., Brockerhoff, E.G., and Gonzalez-olabarria, J.R. (2017). Tree diversity drives forest stand resistance to natural disturbances. Curr. For. Rep. 3, 223–243.

Jucker, T., Bouriaud, O., Avacaritei, D. and Coomes, D.A. (2014). Stabilizing effects of diversity on aboveground wood production in forest ecosystems: linking patterns and processes. Ecol. Lett., 17, 1560–1569.

Kareiva, P., Watts, S., McDonald, R., and Boucher, T. (2007). Domesticated nature: Shaping landscapes and ecosystems for human welfare. Science, 316, 1866–1869.

Kelty, M.J., Cameron, I.R. (1995). Plot designs for the analysis of species interactions in mixed stands. Common. For. Rev. 74, 322–332. Lamb, D., Erskine, P.D., and Parrotta, J.A. (2005). Restoration of degraded tropical forest landscapes. Science 310, 1628–1632.

Kelty, M.J. (2006). The role of species mixtures in plantation forestry. Forest Ecology and Management 233, 195–204.

Lambin, E.F. and Meyfroidt, P. (2011) Global land use change, economic globalization, and the looming land scarcity. PNAS, 108, 3465-3472.

Laforest-lapointe, I., Paquette, A., Messier, C., and Kembel, S.W. (2017). Leaf bacterial diversity mediates plant diversity and ecosystem function relationships. Nature 546, 145–147. Laliberté, E., Legendre, P., 2010. A distance-based framework for measuring functional diversity from multiple traits. Ecology 91, 299–305.

Lebourgeois F., Gomez N., Pinto P., and M´erian P. (2013). Mixed stands reduce Abies alba tree-ring sensitivity to summer drought in the Vosges mountains, western Europe. Forest Ecology and Management, 303, 61–71.

Liang, J., Crowther, T.W., Picard, N., Wiser, S., Zhou, M., and Alberti, G. (2016). Positive biodiversity–productivity relationship predominant in global forests. Science, 354, aaf8957.

Tony Chahine, The potential of Biodiversity for Adaptation Strategies to Global Change in Mediterranean Ecosystems. PhD thesis in Agrometeorology and Ecophysiology of Agricultural and Forest Systems, University of Sassari.

Lloret, F., Escudero, A., Iriondo, J.M., Martínez-Vilalta, J., and Valladares, F. (2012). Extreme climatic events and vegetation: the role of stabilizing processes. Global Change Biology 18, 797–805.

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., and Hector, A. (2001). Biodiversity and ecosystem functioning: current knowledge and future challenges. Science, 294, 804–808.

Loreau, M., Downing, A., Emmerson, M., Gonzalez, A., Hughes, J., and Inchausti, P. (2002). A new look at the relationship between diversity and stability. In: Biodiversity and Ecosystem Functioning: Synthesis and Perspectives (eds Loreau, M., Naeem, S. and Inchausti, P.). Oxford University Press, Oxford, pp. 79–91.

Madrigal-gonzález, J., Ruiz-benito, P., Ratcliffe, S., Calatayud, J., Kändler, G., Lehtonen, A., Dahlgren, J., Wirth, C., and Zavala, M.A. (2016). Complementarity effects on tree growth are contingent on tree size and climatic conditions across Europe. Sci. Rep. 6, 32233.

Magh R.-K., Bonn B., Grote R., Burzlaff T., Pfautsch S., and Rennenberg H. (2019). Drought superimposes the positive effect of silver fir on water relations of European beech in mature forest stands. Forests, 10, 897.

Mason, N.W.H., MacGillivray, K., Steel, J.B. and Wilson, J.B. (2003). An index of functional diversity. J. Veg. Sci., 14, 571–578.

McCann, K.S. (2000). The diversity-stability debate. Nature, 405, 228–233.

Messier C., Bauhus J., Sousa-Silva R., Auge H., Baeten L., Barsoum N., Bruelheide H., Caldwell B., Cavender-Bares J., Dhiedt E., Eisenhauer N., Ganade G., Gravel D., Guillemot J., Hall J.S., Hector A., H´erault B., Jactel H., Koricheva J., Kreft H., Mereu S., Muys B., Nock C.A., Paquette A., Parker J.D., Perring M.P., Ponette Q., Potvin C., Reich P.B., Scherer-Lorenzen M., Schnabel F., Verheyen K., Weih M., Wollni M., and Zemp D.C. (2021). For the sake of resilience and multifunctionality, let's diversify planted forests! Conservation Letters, e12829.

Millennium Ecosystem Assessment. (2005a) Ecosystem and Human Well-Being: Current State and Trends. Island Press, Washington, DC.

Morin, X., L. Fahse, M. Scherer-Lorenzen, and H. Bugmann. (2011). Tree species richness promotes productivity in temperate forests through strong complementarity between species. Ecology Letters 14: 1211–1219.

Nadrowski, K., C. Wirth, and M. Scherer-Lorenzen. (2010). Is forest diversity driving ecosystem function and service? Current Opinion in Environmental Sustainability 2: 75–79.

Nester, M.R. (1994). A Compendium of Designs and Plant Arrangements for the Study and Control of Plant Interactions. Unpublished Report, Queensland Forest Research Institute, Gympie, Australia, 51 pp.

Paquette, A. and Messier, C. (2011). The effect of biodiversity on tree productivity: from temperate to boreal forests. Glob. Ecol. Biogeogr., 20, 170–180.

Pawson, S.M., Brin, A., Brockerhoff, E.G., Lamb, D., Payn, T.W., Paquette, A., and Parrotta, J.a. (2013). Plantation forests, climate change and biodiversity. Biodivers. Conserv. 22, 1203–1227.

Petchey, L., and Kevin Gaston, J. (2006). Functional diversity: back to basics and looking forward. Ecology Letters, 9: 741–758.

Pimm, S.L. (1984). The complexity and stability of ecosystems. Nature, 307, 321–326.

Piotto, D., Viquez, E., Montagnini, F., and Kanninen, M. (2004). Pure and mixed forest plantations with native species of the dry tropics of Costa Rica: a comparison of growth and productivity. For. Ecol. Manage. 190, 359– 372.

Powers, R.F. (1999). On the sustainable production of planted forests. New For. 17, 263–306.

Pretzsch H., Dieler J., Seifert T., and R¨otzer T. (2012) Climate effects on productivity and resource-use efficiency of Norway spruce (Picea abies [L.] Karst.) and European beech (Fagus sylvatica [L.]) in stands with different spatial mixing patterns. Trees, 26, 1343–1360.

Pretzsch, H., Schütze, G,. and Uhl, E. (2013b). Resistance of European tree species to drought stress in mixed versus pure forests: evidence of stress release by inter-specific facilitation. Plant Biol., 15, 483–495.

Pretzsch H., R̈otzer T., Matyssek R., Grams T.E.E., Ḧaberle K.-H., Pritsch K., Kerner R., and Munch J.-C. (2014) Mixed Norway spruce (*Picea abies* [L.] Karst) and European beech (Fagus sylvatica [L.]) stands under drought: from reaction pattern to mechanism. Trees, 28, 1305–1321.

Potvin, C., and Gotelli, N.J. (2008). Biodiversity enhances individual performance but does not affect survivorship in tropical trees. Ecol. Lett. 11, 217–223. http://dx.doi.org/10. 1111/j.1461-0248.2007.01148. x.

Ratcliffe, S., Liebergesell, M., Ruiz Benito, P., Madrigal González, J., Muñoz Castañeda, J.M., and Kändler, G. (2016). Modes of functional biodiversity control on tree productivity across the European continent. Glob. Ecol. Biogeogr., 25, 251–262.

Richards, A.E., Forrester, D.I., Bauhus, J., and Scherer-Lorenzen, M. (2010). The influence of mixed tree plantations on the nutrition of individual species: a review. Tree Physiol., 30, 1192–1208.

Ricotta, C. (2005). A note on functional diversity measures. Basic Appl. Ecol., 6, 479–486.

Rockström, B.J., Gaffney, O., Rogeli, J., Meinshausen, M., Nakicenovic, N., and Joachim, H. (2017) A roadmap for rapid decarbonization. Science, 355, 1269-1271.

Rodrı́guez-Robles U., Arredondo J.T., Huber-Sannwald E., Y´epez E.A., and Ramos-Leal J.A. (2020) Coupled plant traits adapted to wetting/drying cycles of substrates co-define niche multidimensionality. Plant, Cell and Environment, 43, 2394–2408.

Roscher, C., Schumacher, J., Baade, J., Wilcke, W., Gleixner, G., and Weisser, W.W. (2004). The role of biodiversity for element cycling and trophic interactions: and experimental approach in a grassland community. Basic Appl. Ecol., 5, 107–121.

Rothe, A., Binkley, and D. (2001). Nutritional interactions in mixed species forests: a synthesis. Can. J. For. Res. 31, 1855–1870.

Sackville Hamilton, N.R. (1994). Replacement and additive designs for plant competition studies. J. Appl. Ecol. 31, 599–603.

Scherer-Lorenzen, M. (2014). The functional role of biodiversity in the context of global change. In Forests and global change, ed. D. Burslem, D. Coomes, and W. Simonson, 195–238. Cambridge: Cambridge University Press.

Schume H., Jost G., and Hager H. (2004) Soil water depletion and recharge patterns in mixed and pure forest stands of European beech and Norway spruce. Journal of Hydrology, 289, 258–274.

Stevens, R.D., Cox, S.B., Strauss, R.E., and Willig, M.R. (2003). Patterns of functional diversity across an extensive environmental gradient: vertebrate consumers, hidden treatments and latitudinal trends. Ecol. Lett., 6, 1099–1108.

Thomas, S., P. Dargusch, S. Harrison, and J. Herbohn. (2010). Why are there so few afforestation and reforestation Clean Development Mechanism projects? Land Use Policy 27: 880–887.

Tilman, D., Isbell, F., and Cowles, J.M. (2014). Biodiversity and ecosystem functioning. Annu. Rev. Ecol. Evol. Syst. 45, 471–493.

Tilman, D. (1996). Biodiversity: population versus ecosystem stability. Ecology, 77, 350–363.

Tilman, D., Reich, P.B., and Knops, J.M.H. (2006). Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature, 441, 629–632.

Tilman, D. (2001). Functional diversity. In: Encyclopaedia of Biodiversity (ed. Levin, S.A.). Academic Press, San Diego, CA, pp.109–120.

Tilman, D. (1999). The ecological consequences of changes in biodiversity: a search for general principles. Ecology, 80, 1455–1474.

Thompson, I., Mackey, B., McNulty, S., and Mosseler, A. (2009). Forest Resilience, Biodiversity, and Climate Change. A synthesis of the biodiversity/resilience/stability relationship in forest ecosystems. Secretariat of the Convention on Biological Diversity, Montreal. Technical Series No. 43.

United Nations. (2017) The 2017 Revisions: World Population Prospects.

Vanclay, J.K. (2006a). Spatially explicit competition indices and the analysis of mixed-species plantings with the Simile modelling environment. For. Ecol. Manage. 233, 295–302.

Vanclay, J.K. (2006b). Experiment designs to evaluate inter- and intra-specific interactions in mixed plantings of forest trees. For. Ecol. Manage. 233, 366–374.

Van de Peer, T., Verheyen, K., Baeten, L., Ponette, Q., and Muys, B. (2016). Biodiversity as insurance for sapling survival in experimental tree plantations. J. Appl. Ecol. 53,1777–1786.

Verheyen, K., Vanhellemont, M., Auge, H., Baeten, L., Baraloto, C., Barsoum, N., Bilodeau Gauthier, S., Bruelheide, H., Castagneyrol, B., Godbold, D., Haase, J., Hector, A., Jactel, H., Koricheva, J., Loreau, M., Mereu, S., Messier, C., Muys, B., Nolet, P., Paquette, A., Parker, J., Perring, M., Ponette, Q., Potvin, C., Reich, P., Smith, A., Weih, M., and Scherer-Lorenzen, M. (2015). Contributions of a global network of tree diversity experiments to sustainable forest plantations. Ambio 45, 29–41.

Vellend, Mark, Lander Baeten, Antoine Becker-Scarpitta, Véronique Boucher-Lalonde, Jenny L. McCune, Julie Messier, Isla H. Myers-Smith, and Dov F. Sax. (2017). Plant Biodiversity Change Across Scales During the Anthropocene. Annual Review of Plant Biology 68 (April). Annual Reviews Inc.: 563–86. doi:10.1146/annurev-arplant-042916-040949.

Watt, A.D. (1992). Insect pest population dynamics: effects of tree species diversity. In: Cannell, M.G.R., Malcolm, D.C., Robertson, P.A. (Eds.), The Ecology of Mixed-Species Stands of Trees. Blackwell Scientific Publications, Oxford, pp. 267–275.

Weiher, E., Clarke, G.D.P., and Keddy, P.A. (1998). Community assembly rules, morphological dispersion, and the coexistence of plant species. Oikos, 81, 309–322.

William F. Laurance, Jeffrey Sayer, and Kenneth G. Cassman. (2014) Agricultural expansion and its impacts on tropical nature, Trends in Ecology and Evolution, Volume 29, Issue 2.

Woods, A., K.D. Coates, and A. Hamann. (2005). Is an unprecedented dothistroma needle blight epidemic related to climate change? BioScience 55: 761–769.

Tony Chahine, The potential of Biodiversity for Adaptation Strategies to Global Change in Mediterranean Ecosystems. PhD thesis in Agrometeorology and Ecophysiology of Agricultural and Forest Systems, University of Sassari.

Yang, B., Li, Y., Ding, B., and Both, S. (2017). Impact of tree diversity and environmental conditions on the survival of shrub species in a forest biodiversity experiment in subtropical China. J. Plant Ecol. 10, 179–189.

Yang, X., Bauhus, J., Both, S., Fang, T., Härdtle, W., Kröber, W., Ma, K., Nadrowski, K., Pei, K., Scherer-Lorenzen, M., Scholten, T., Seidler, G., Schmid, B., von Oheimb, G., and Bruelheide, H. (2013). Establishment success in a forest biodiversity and ecosystem functioning experiment in subtropical China (BEF-China). Eur. J. For. Res. 132, 593–606. http://dx.doi.org/10.1007/s10342-013-0696-z.

Zhang Y., Chen H.Y.H., and Reich P.B. (2012). Forest productivity increases with evenness, species richness and trait variation: a global meta-analysis. Journal of Ecology, 100, 742–749.

II. Chapter 2 Effects of tree species diversity and water availability on insect herbivory in *Quercus ilex* L.

II.1 Abstract

Quercus ilex L., a resilient evergreen oak species, faces complex interactions between drought stress and insect herbivory, which are critical factors influencing its ecological dynamics and tree health. Two experiments in different biomes (Temperate and Mediterranean) were conducted to investigate the factors influencing herbivory on *Q. ilex*, with a focus on the effects of neighboring tree species, species richness (SR), and irrigation treatments. We hypothesized that herbivory would be highest when *Quercus ilex* L. trees were surrounded by trees of the same species and decrease as species richness and functional diversity of neighboring trees increased. Additionally, we expected higher herbivory in irrigated blocks due to potentially lower defense mechanisms. We also examined the role of pine trees as neighboring species, as they may hide *Q. ilex* from herbivores. Our results indicated that species richness and irrigation treatment did not significantly affect herbivory, which was instead significantly different between the ORPHEE and IDENT-MACOMER sites. Herbivory on *Q. ilex* was consistently lower in the ORPHEE site, irrespective of species richness. Irrigation had no significant impact on herbivory in either site. Furthermore, the presence of pine trees significantly increased herbivory levels, suggesting an interaction between pine trees and herbivorous insects. The complex interplay between local site conditions, species diversity, and herbivory highlights the importance of considering these factors for effective forest management and conservation strategies.

The results underscore the multifaceted nature of herbivory dynamics in mixed forests, with potential influences ranging from species interactions to microclimatic conditions. Understanding these dynamics is crucial for the management and preservation of mixed forests in the face of changing environmental condition

Quercus ilex L.; drought stress; insect herbivory; ecological dynamics; tree diversity

II.2 Introduction

The intricate balance of forest ecosystems, sculpted over millennia, reflects a symphony of interactions between the biotic and abiotic realms. Among these myriad interactions, the relationship between tree diversity and insect herbivory has emerged as an area of significant ecological intrigue. Indeed, early ecological literature, beginning with pioneers such as Root (1973), has pointed towards the profound influence tree diversity wields over herbivore populations and their feeding behaviors. Within this complex web, an intriguing pattern emerges: as plant diversity augments, herbivory on focal plant species frequently diminishes, a relationship termed 'associative resistance' (Letourneau et al., 2011). This phenomenon, evidenced across diverse landscapes from agricultural fields to dense forests, underscores the potentially protective effects of a diverse plant community.

However, while the protective shield of 'associative resistance' has been identified in numerous controlled studies, extrapolating these findings to more unpredictable, real-world scenarios presents challenges (Schuldt et al., 2010). Forests, with their inherent dynamism, are subjected to a multitude of variables ranging from tree species composition, age classes, spatial arrangement, to overarching climatic conditions. Predicting herbivory in such intricate systems necessitates a deeper understanding of the mechanisms driving these interactions.

The holm oak (*Quercus ilex* L.), a flagship species of the Mediterranean biome, known for its resilience to multiple stresses and its importance as a bastion for various herbivorous insects. While the relationships between tree diversity and herbivory have been relatively well explored in more temperate and tropical settings, *Q. ilex* represents an exciting and relatively uncharted frontier. This iconic species, with its robust growth and diverse suite of defensive compounds, provides an ideal backdrop against which to dissect the nuances of herbivory in the face of varying tree diversity and environmental conditions (Castagneyrol et al., 2014).

Another layer of complexity is introduced when one considers water availability. Water, a key determinant in plant physiology, not only influences growth but can also modulate plant defenses as well as insect population dynamics. Previous research on various plant species suggests that water stress might either amplify or attenuate a plant's defensive repertoire, contingent on the specific ecological context (Tariq et al., 2013). Given the increasing unpredictability of water availability due

to climate change, particularly in Mediterranean regions, understanding its interplay with tree diversity becomes paramount.

Considering the aforesaid details, our study seeks to unravel the combined and individual effects of tree species composition and water availability on the growth, defenses, and herbivore interactions of *Q. ilex*. Through our exploration, we hope to contribute a nuanced understanding that could serve as a beacon for both ecologists and foresters navigating the challenges of a rapidly changing world.

In the context of shifting global climate patterns and increased anthropogenic impacts, exploring the factors that influence insect herbivory on *Q. ilex* emerges as a pressing endeavor (Vila et al., 2007). This study delves into the multifaceted relationship between tree species diversity and water availability, and their collective impact on insect herbivory in *Q. ilex* stands. By dissecting the interactions between these variables, we aim to shed light on the complex mechanisms that underpin these relationships, providing insights that can inform forest management strategies and conservation efforts (Diaz et al., 2007; Jactel et al., 2019).

In the present study we hypothesized that herbivory on *Q. ilex* would be highest when the target tree was neighbored by trees of the same species while it would decrease as the number of species and or functional diversity of the neighboring trees would increase. We also hypothesized that herbivory would be higher in irrigated blocks as compared to not irrigated ones possibly because the defense mechanisms in irrigated blocks would be lower. Finally, we tested if besides the effect of SR and FDis, herbivory on *Q. ilex* could be predicted by the presence of pines as neighboring trees as the Pines are fast growing species that tend to dominate, at least in the first stages of stand development, and could "hide" *Q. ilex* individuals from herbivores.

II.3 Materials and Methods

The holm oak is a slow-growing, evergreen species known for its shade tolerance, primarily found in the central-western Mediterranean basin (Rodà et al. 1999, De Rigo and Caudullo 2016). Its natural range is largely confined to the Mediterranean basin, stretching from Portugal to Turkey, with some populations along the Atlantic coast of France. This versatile species thrives in various habitats, ranging from mixed forests and scrublands to pure stands, and can adapt to diverse climates, from semi-arid to highly humid conditions. Notably, *Q. ilex* is a sclerophyllous plant, characterized by dark, leathery leaves with a white-haired underside that reduces transpiration, enhancing its resilience to drought. However, it has been observed that in situations of severe water stress, such as competition with other deciduous oaks and conifers, Q. ilex trees may even suspend their vegetative activities during dry spells (Barbero et al. 1992, De Rigo and Caudullo 2016).

Throughout its distribution, a wide range of both specialist and generalist insect herbivores are known to feed on this species. These herbivores include leaf chewers, miners, and skeletonizes (Southwood et al. 2005, Ruiz-Carbayo et al. 2017, Hernández-Agüero et al. 2022). In response to these herbivorous attacks, Q. ilex has developed an array of secondary metabolites within its leaves. These compounds serve as potential defenses against various biotic and abiotic stressors, encompassing phenolic compounds (Moreira et al. 2019b, 2020) and volatile organic compounds (VOCs) (Holzinger et al. 2000). While the role of VOCs as indirect defenses in Q. ilex has yet to be fully explored, they have been shown to attract natural enemies of herbivores in other Quercus species (Ghirardo et al. 2012, Pearse et al. 2013).

Two sites Explored: Sardinia and Bordeaux

During this comparative ecological investigation, our focus extends to two ecologically distinct biomes: the Mediterranean biome, embodied by the island of Sardinia in Italy, and the temperate biome, which finds its model in the Bordeaux region of France. These two biomes, each exemplifying a distinct ecological paradigm, offer a rich tapestry of features that set them apart. The Mediterranean biome, characterized by a Mediterranean climate, epitomizes Sardinia, boasting key characteristics such as warm, arid summers and mild, rainy winters. In this biome, one encounters a unique assembly of flora, including but not limited to drought-resistant plants, evergreen shrubs, and iconic species like the holm oak (*Quercus ilex* L.). Biodiversity thrives here, with a multitude of species adapted to the region's climatic patterns and ecological dynamics, rendering Sardinia a vivid mosaic of ecological diversity.

On the other hand, the temperate biome, symbolized by Bordeaux in France, unveils an entirely different set of environmental attributes. It typifies the temperate biome with its four-season climate, characterized by warm summers and cold winters. Bordeaux, situated in the southwest of France, is subject to a temperate maritime climate heavily influenced by its proximity to the Atlantic Ocean. The vegetation in this biome is emblematic of deciduous forests, marked by the seasonal leaf-shedding of a variety of tree species, prominently including oaks, beeches, and pines. Biodiversity in the temperate biome of Bordeaux reflects adaptation to the distinct seasonal fluctuations and temperate climatic conditions, rendering the region renowned for its diverse ecosystems, encompassing both terrestrial and aquatic habitats.

A comprehensive understanding of the unique attributes of the Mediterranean and temperate biomes serves as an essential prelude to elucidating the outcomes of the experiments conducted within these disparate ecological settings, which will be elaborated upon in the ensuing sections. This comparative approach enables us to explore how ecological interactions and responses manifest differently in these contrasting environments, shedding light on the intricate dynamics that govern these distinct biomes.

Description of the experiments:

II.4 Experiment 1: IDENT-MACOMER

IDENT-MACOMER is located on the island of Sardinia (Italy), Macomer (40° 14' N; 8° 42' E) 640m above sea level within the nursery "Saint Antonio - Sardinian Forest Authority". It is part of the International Diversity Experiment Network with Trees (IDENT), (Tobner et al. 2014). The hot-summer Mediterranean climate (Köppen: Csa) is characterized by warm and dry summers and mild winters with moderate rainfall. In Macomer, the hot season lasts for about 3 months, from June 15 to September 9, with an average daily high temperature above 26°C. The hottest month of the year in is July, with an average high of 29°C and low of 16°C; the cool season lasts for 4 months, from November 18 to March 20, with an average daily high temperature below 14°C. The coldest month of the year in Macomer is January, with an average low of 1°C and high of 10°C (Figure 1).

Average	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
High	10°C	11°C	14°C	17°C	22°C	26°C	29°C	29°C	25°C	21°C	15°C	11°C
Temp.	5°C	5°C	8°C	11°C	15°C	20°C	23°C	23°C	19°C	15°C	10°C	6°C
Low	1°C	1°C	3°C	5°C	9°C	13°C	16°C	16°C	13°C	9°C	5°C	2°C
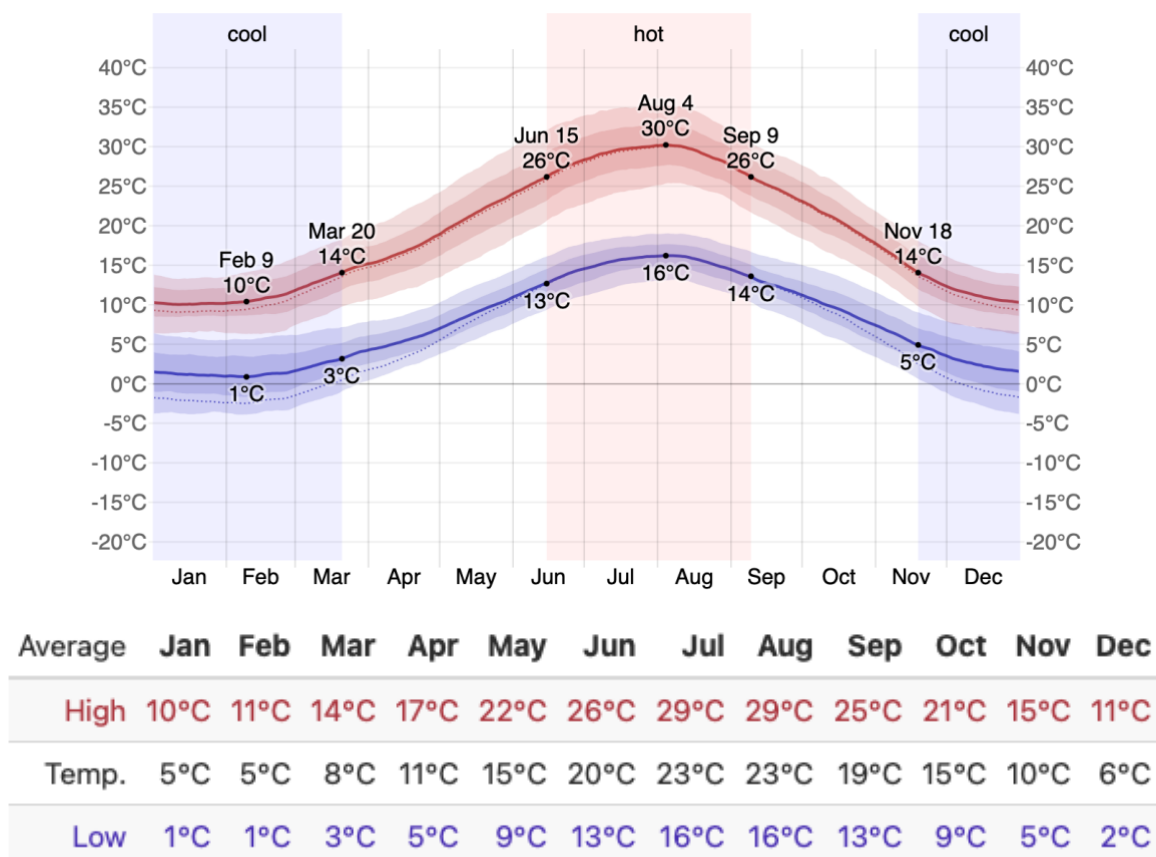
Figure 1. *The daily average high (red line) and low (blue line) temperature, with 25th to 75th and 10th to 90th percentile bands. The thin dotted lines are the corresponding average perceived temperatures (https://weatherspark.com/y/58815/Average-Weather-in-Macomer-Italy-Year-Round#Figures-PrecipitationProbability).*

IDENT-MACOMER experiences significant seasonal variation in monthly rainfall (Figure 2), the rainfall accumulated over a sliding 31-day period centered around each day of the year. The rainy period of the year lasts for 9.3 months, from August 27 to June 6, with a sliding 31-day rainfall of at least 13 millimeters. The month with the most rain is November, with an average rainfall of 57 millimeters.

The rainless period of the year lasts for 2.6 months, from June 6 to August 27. The month with the least rain is July, with an average rainfall of 3 millimeters.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Rainfall	29.5mm	31.5mm	27.6mm	30.4mm	23.7mm	8.8mm	3.2mm	6.7mm	28.3mm	45.0mm	57.0mm	41.1mm
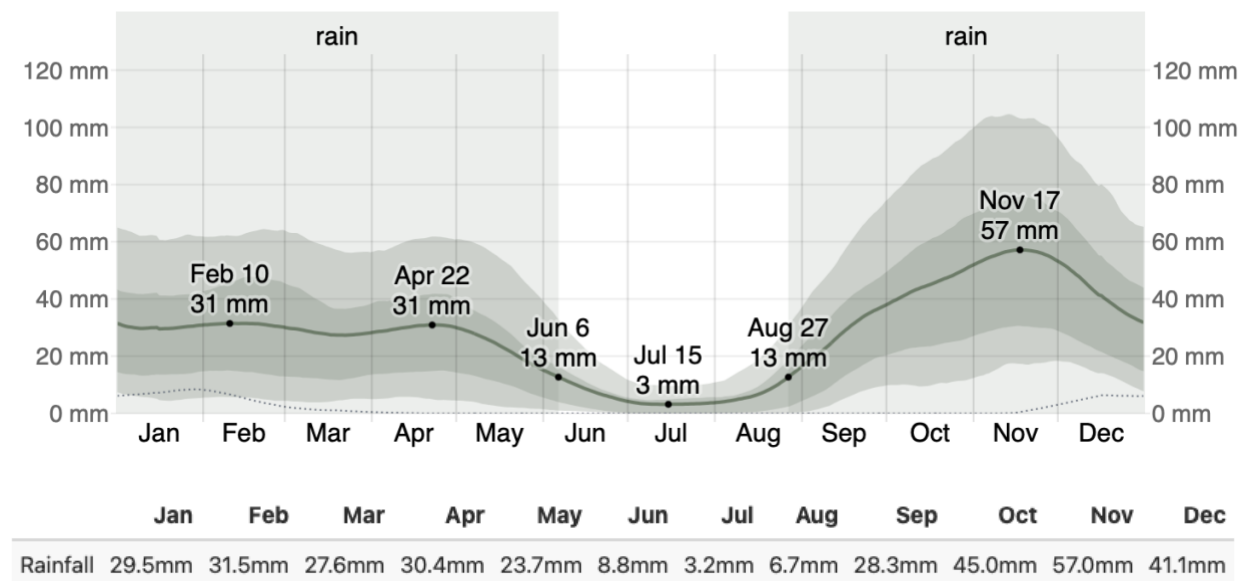
Figure 2. *The average rainfall (solid line) accumulated over the course of a sliding 31-day period centered on the day in question, with 25th to 75th and 10th to 90th percentile bands. The thin dotted line is the corresponding average snowfall (https://weatherspark.com/y/58815/Average-Weather-in-Macomer-Italy-Year-Round#Figures-PrecipitationProbability),*

The precipitation patterns in Macomer (Figure 3) exhibit distinct wet and dry seasons throughout the year. A wet day, defined as one with at least 1 mm of liquid or liquid-equivalent precipitation, is prevalent during the wetter season, spanning 7.5 months from September 25 to May 9, where there is a greater than 16% chance of daily precipitation. November emerges as the wettest month, averaging 8.6 days with at least 1 mm of precipitation. Conversely, the drier season encompasses 4.5 months from May 9 to September 25, with July being the driest month, experiencing an average of only 0.7 days with significant precipitation. Further analysis distinguishes between precipitation types, revealing that rain alone is the predominant form throughout the year, reaching its peak probability of 31% on November 24. These intricate precipitation dynamics underscore the seasonality and variability in Macomer's climatic conditions.
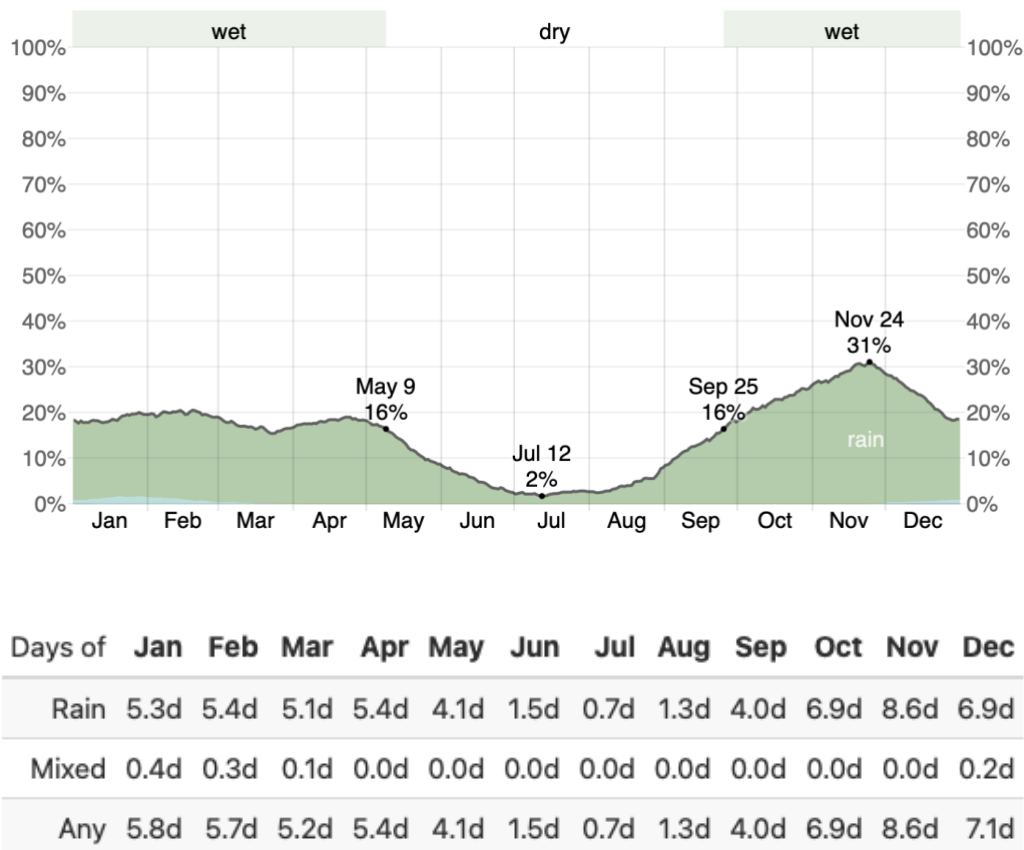
Figure 3. *The percentage of days in which various types of precipitation are observed, excluding trace quantities: rain alone, snow alone, and mixed (both rain and snow fell in the same day). (https://weatherspark.com/y/58815/Average-Weather-in-Macomer-Italy-Year-Round#Figures-PrecipitationProbability)*

The basaltic area was used as a tree nursery for local reforestation projects until 2008. Up to 2014 (the establishment of the experiment) the fields were left unmanaged, although spontaneous vegetation (grasses) was periodically removed. In 2015 a meteorological station was placed in an open field adjacent to the experiment.

The station measures precipitation, photosynthetic active radiation, wind speed and wind direction at 2 m height, as well as air temperature and relative humidity at 0.3. 2 and 4 m height. All sensors were connected to a data logger acquiring data every 5 minutes and storing them as 30 minutes averages, or sum for precipitations.

In line with other IDENT network sites, the experimental design features a hierarchical structure, arranging trees within 308 plots distributed over seven blocks (Figure 4). A block comprises 44 plots of 3.2 m by 3.2 m, with in each plot 64 seedlings planted at 40 cm spacing. Blocks are exact replicates in terms of tree species communities in the plots, but the spatial arrangement of plots within blocks is random. All blocks were irrigated during the dry season in the first year (2014). In the following years, three selected blocks were irrigated with 20 mm every 15 days from June to September.

For this a pipe system was used to distribute water homogeneously over the soil. The remaining four blocks were not irrigated; thus, they serve as controls (Figure 1). From the end of January to mid-April 2014, there were 19,712 containerized one-year- old. Species selection was based on site requirements and considering local availability of seedlings in the nursery.

In total, 12 native woody species (Table 1) were selected, of which both shrubs (three species) and trees (nine species), and among them three conifers (*Pinus halepensis* Mill*., Pinus pinaster* Ait., *Pinus pinea* L.), six evergreen broad-leaved species and three deciduous broad-leaved species (*Acer monspessulanum* L*., Arbutus unedo* L*., Fraxinus ornus* L*., Olea europea* L*., Phillyrea latifolia* L*., Pistacia lentiscus* L*., Quercus ilex* L*., Quercus pubescens* Willd*.* and *Quercus suber* L.). Within each block, a first diversity gradient was created by manipulating SR at four levels: one (12 plots), two (17 plots), four (9 plots) and six- species (6 plots). A second gradient, orthogonal to the first, was created by manipulating FD.

Figure 4. *Experimental design of IDENT-MACOMER (Sardinia, Macomer), a single-site tree diversity experiment created in 2014. The experiment is hinges on a high-density planting design (0.4 m by 0.4 m) and small plot sizes (10.2 m2). The planting corresponds to densities of regenerating forests and should speed-up the onset of tree-tree interactions (Tobner et al. 2014). The small plot size allows more plot replicates, although it is at the cost of a plot's scientific lifespan. On top of the figure, a site plan is shown with three irrigated (grey) and four non-irrigated (white) blocks, which allows testing the stress gradient hypothesis. In the middle left of the figure, the disposition of 44 plots within a block, including orthogonal gradients of SR and FD is shown; In the middle-right there is the arrangement of 64 plants in a four-species plot. Bold lines represent inner, middle and outer frame. Species relative abundances within these frames are alike and the outer frame was not included in the analyses to avoid plot edge effects. A broad species pool of 12 site-adapted Mediterranean species (including conifers, deciduous broadleaved and evergreen broadleaved species) was used to create the monocultures and mixed communities (lower part). All plots were regularly weeded in the first three years to avoid overgrow by herbaceous vegetation.*

Table 1. *Taxonomic Classification, Common Nomenclature, and Leaf Habit Characteristics of Studied Tree Species (IDEENT-M).*

Latin name	Common name	Leaf habit
Quercus ilex L.	Holm Oak	Evergreen
Quercus suber L.	Cork Oak	Evergreen
Quercus pubescens Willd.	Downy Oak	Deciduous (Marcescent)
Pinus pinaster Ait.	Maritime Pine	Evergreen
Pinus pinea L.	Stone Pine	Evergreen
Pinus halepensis Mill.	Aleppo Pine	Evergreen
Phillyrea latifolia L.	Green Olive Tree	Evergreen
Acer monspessulanum L.	Montpellier Maple	Deciduous
Pistacia lentiscus L.	Mastic Tree	Evergreen
Arbutus unedo L.	Strawberry Tree	Evergreen
Fraxinus ornus L.	Manna Ash	Deciduous
Olea europea L.	Olive Tree	Evergreen

II.5 Experiment 2: ORPHEE

ORPHEE (Observatoire Régional de la Phénologie / Regional Phenology Observatory) experiment is located 40 km south of Bordeaux, France (44° 440 N, 00° 460 W), which belongs to the worldwide Tree Diversity Network (TreeDivNet1). The experimental plantation was established in 2008 on a clear cut of former maritime pine stands. Stumps were removed and the site, on a sandy podzol, was ploughed and fertilized with phosphorus and potassium before planting. In total, 25 600 trees of five native species (Table 2) were planted (*Betula pendula* Roth, *Quercus robur* L., *Quercus pyrenaica* Willd., *Quercus ilex* L. and *Pinus pinaster* Ait.) within a 12-ha area (Figure 5). Eight blocks were established, with 32 plots in every block, corresponding to the 31 possible combinations of one to five species, with an additional replicate of the combination of five species. Each plot contained 10 rows of 10 trees planted 2 m apart, resulting in 100 trees per plot, with a plot area of 400 m^2. Tree species mixtures were established according to a substitutive design, keeping tree density equal across plots. Within plots, individual trees from different species were planted in a regular alternate pattern, such that a tree from a given species had at least one neighbor from each of the other species within a 2 m radius. The understory vegetation was mowed once per year. Plots were separated by three meters and were randomly distributed within blocks. Blocks covered an area of 100 x 175 m, and the entire experimental site was fenced to prevent grazing by mammalian herbivores.
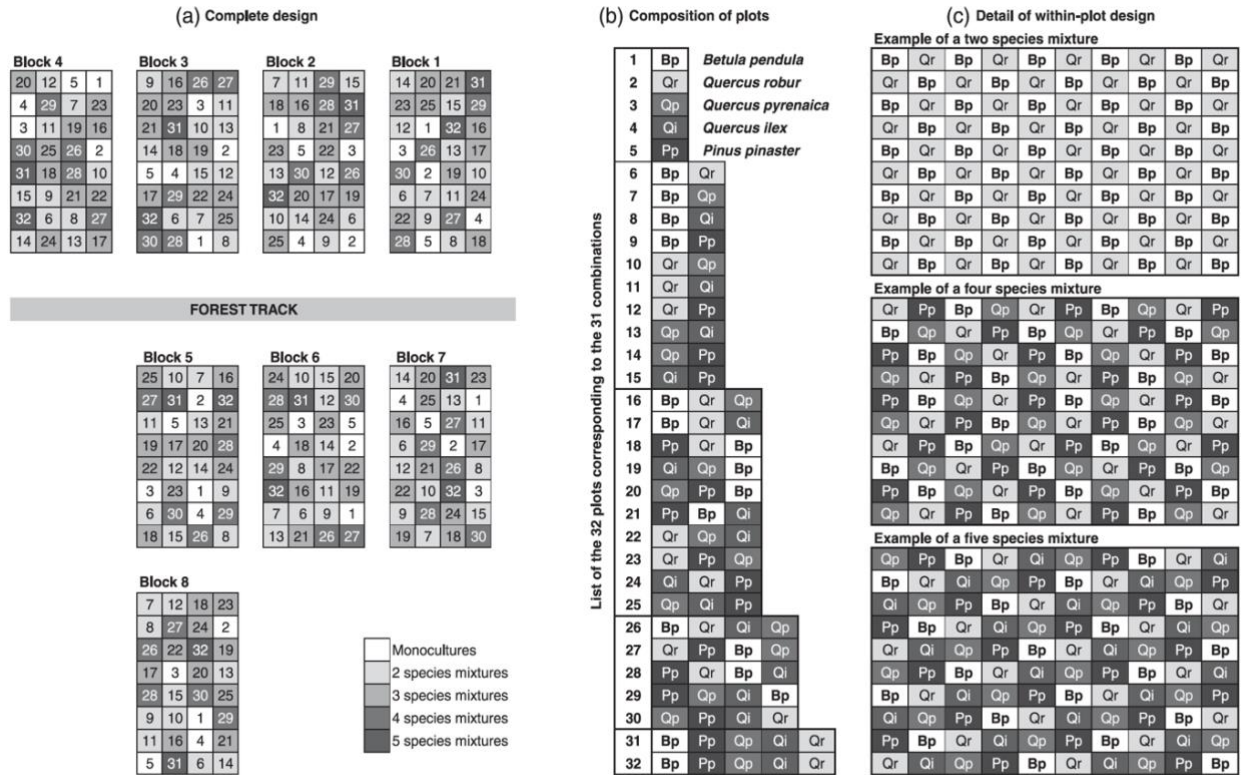
Figure 5. *Experimental design of ORPHEE (France, Bordeaux) consisted of eight blocks and 32 plots within each block. Each plot represented a tree species composition treatment, corresponding to 31 possible combinations of one to five tree species (Betula pendula Roth, Quercus robur L., Quercus pyrenaica Willd., Quercus ilex L. and Pinus pinaster Ait.) and an additional plot replicate of the five species mixture. Each plot contained 10 rows of 10 trees planted 2 m apart (100 trees on 400 m2). Tree species mixtures were established according to a substitutive design, keeping tree density of tree neighbors equal across plots. Within plots, individual trees from different species were planted in a regular alternate pattern, such that a tree from a given species had at least one neighbor from each of the other species within a 2-m radius.*

Table 2. *Taxonomic Classification, Common Nomenclature, and Leaf Habit Characteristics of Studied Tree Species (ORPHEE).*

Latin name	Common name	Leaf habit
Betula pendula Roth	Silver Birch	Deciduous
Quercus robur L.	English Oak	Deciduous
Quercus pyrenaica Willd.	Pyrenean Oak	Evergreen
Quercus ilex L.	Holm Oak	Evergreen
Pinus pinaster Ait.	Maritime Pine	Evergreen

In Bordeaux, the annual temperature pattern reveals distinct warm and cool seasons (Figure 6). The warm season extends for 3.2 months, commencing on June 10 and concluding on September 17, characterized by daily high temperatures consistently exceeding 24°C. July emerges as the hottest month, featuring an average high of 27°C and a low of 15°C. Conversely, the cool season spans 3.5 months, from November 16 to March 2, during which daily high temperatures consistently fall below 13°C. January stands out as the coldest month, with an average low of 3°C and a high of 10°C.

Average	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
High	10°C	11°C	15°C	17°C	21°C	24°C	27°C	27°C	24°C	19°C	14°C	10°C
Temp.	6°C	7°C	10°C	12°C	16°C	19°C	21°C	21°C	18°C	14°C	9°C	7°C
Low	3°C	3°C	5°C	7°C	10°C	13°C	15°C	15°C	12°C	9°C	6°C	3°C
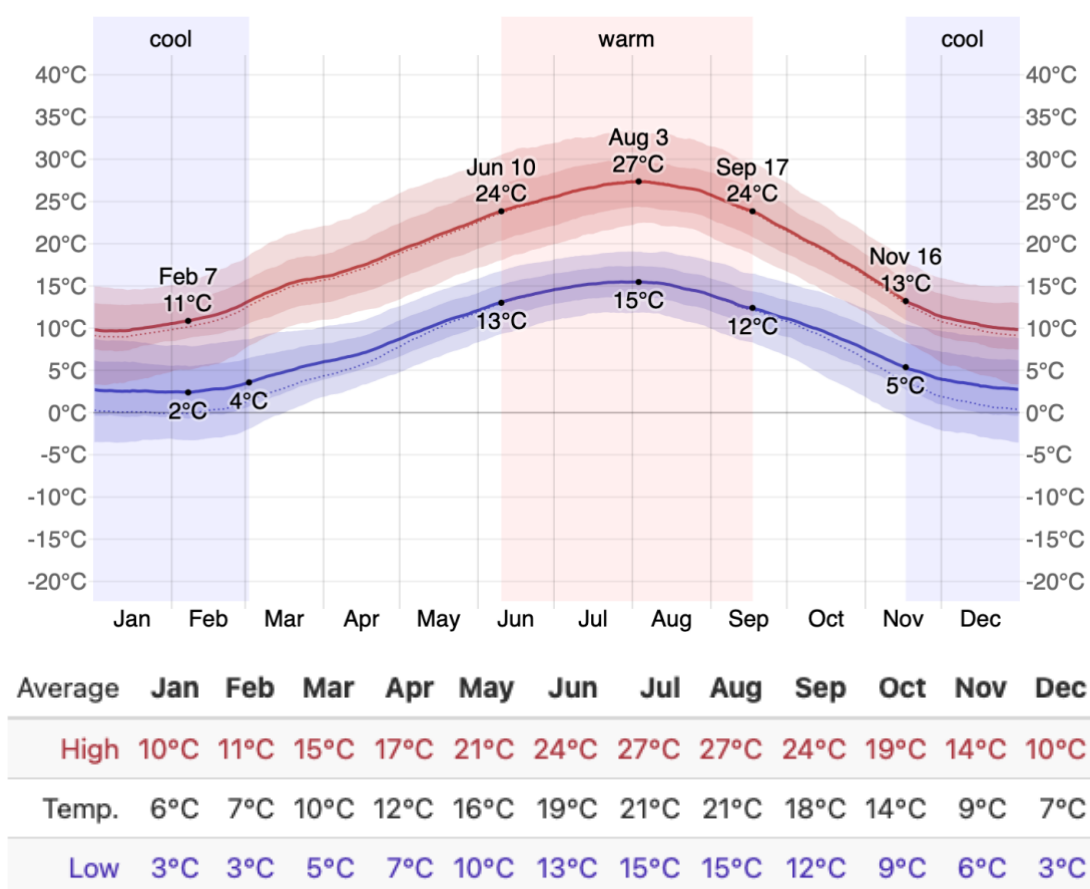
Figure 6. *The daily average high (red line) and low (blue line) temperature, with 25th to 75th and 10th to 90th percentile bands. The thin dotted lines are the corresponding average perceived temperatures (https://weatherspark.com/y/43607/Average-Weather-in-Cestas-France-Year-Round#Figures-Rainfall).*

The rainfall (Figure 7) accumulated over a sliding 31-day period centered around each day of the year. Bordeaux experiences some seasonal variation in monthly rainfall and rain falls throughout the year. The month with the most rain is November, with an average rainfall of 71 mm, the month with the least rain is July, with an average rainfall of 30 mm.
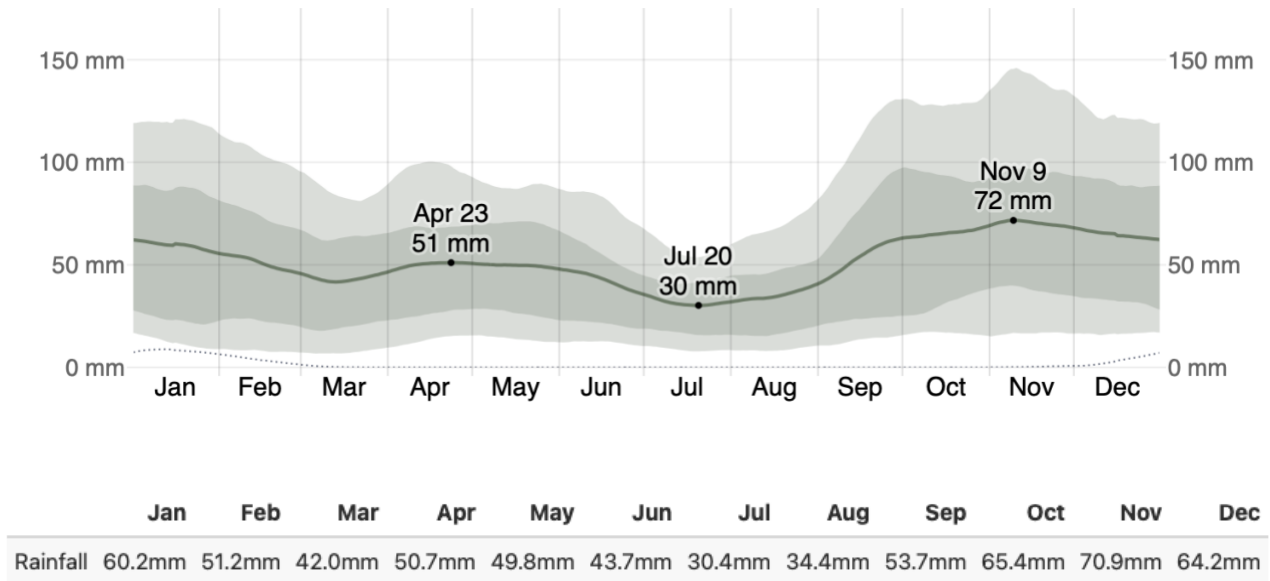
Figure 7. *The average rainfall (solid line) accumulated over the course of a sliding 31-day period centered on the day in question, with 25th to 75th and 10th to 90th percentile bands. The thin dotted line is the corresponding average snowfall*
(https://weatherspark.com/y/43607/Average-Weather-in-Cestas-France-Year-Round#Figures-Rainfall).

The precipitation patterns in Bordeaux (Figure 8) during the fall season is defined as one with at least 1 mm of liquid or liquid-equivalent precipitation. Notably, the likelihood of encountering a wet day experiences a substantial and swift increase throughout the fall season, initiating at a 20% chance and concluding at 31%. For reference, the highest daily probability of a wet day within the entire year is recorded at 34% on November 17, while the lowest likelihood is observed on August 24 at 17%.
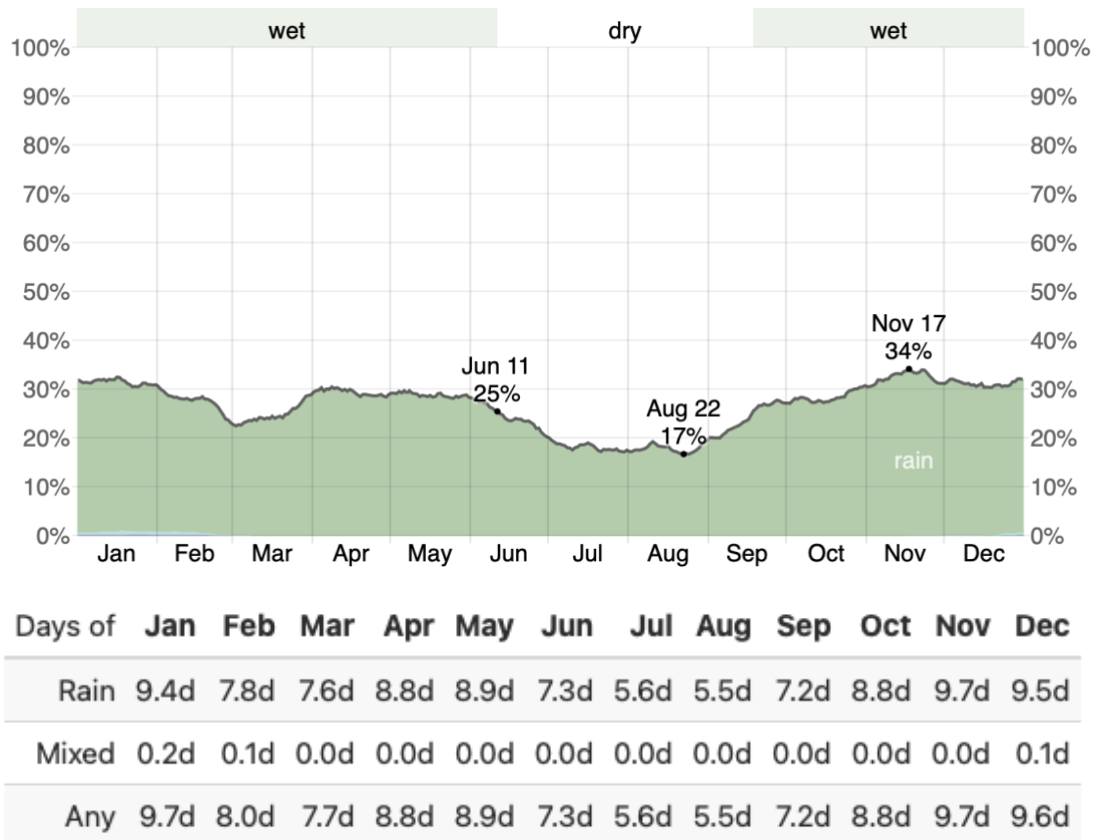
Figure 8. *The percentage of days in which various types of precipitation are observed, excluding trace quantities: rain alone, snow alone, and mixed (both rain and snow fell in the same day). (https://weatherspark.com/y/43607/Average-Weather-in-Cestas-France-Year-Round#Figures-Rainfall).*

II.6 Leaf sampling and herbivory assessment on *Quercus ilex* L.

Leaf sampling has started in July 2021, we concentrated on the plots that contains *Q. ilex* in monoculture with its combinations of different species. In each plot, only saplings in the core area were sampled to avoid edge effects (edge sampling was an option when there were not enough living trees in the center part).

The fixed number of trees per species was assessed regardless of the composition of trees in the plot, six trees were chosen at random in monoculture plots and three trees in 2 species composition.

From each tree, a total of 20 leaves were collected through sampling, which involved gathering 10 leaves from the upper canopy and 10 from the lower canopy. For each canopy position, the sampling included 5 external leaves from the branch tips and 5 internal leaves closer to the trunk (the closest ones). Most likely in some individual we modified it depending on the size and number of leaves in each tree (to avoid the fresh young leaf at the top of each branch). In some cases, few branches hadn't leaves so we have chosen another branch at the same height or from the main axis, the samples were stored in labeled paper bags then transported to the laboratory for analysis.

A total of 9,480 leaves were surveyed in the two experiments, insect herbivores was assessed visually in the laboratory by estimating the percentage of damage on each leaf using the following classes: zero = no damage; 1 = 1–5% damaged; 2 = 6–10% damaged; 3 =11–25% damaged; 4 = 26–50% damaged; 5 = 51–75% damaged; 6 ≥ 75% damaged. We averaged class values across all leaves to obtain a mean value per tree for statistical analyses. A subset of 6 leaves was selected with little or no evidence of herbivory for further chemical analyses of phenolic compounds. The leaves were dried in the oven for 48 h at 45°C.

II.7 Data analysis

Mixed models were used to assess the effects of site, irrigation, species Richness (SR), functional dispersion (FDis) and Community-Weighted Mean (CWM) traits on insect herbivory using % Leaf Area Removed (LAR) as the response variable. % LAR was logged transformed before analysis to improve the normality of the distribution. Blocks and plots were used as nested random effects.

Species values for Specific Leaf Area (SLA), Maximum Height (Mh), Seed Dry Weight (SDW), Wood Density (WD) were estimated by averaging all data found in the TRY Plant Trait Database (TRY Plant Trait Database (try-db.org) for the species of the two experiments. A categorical leaf habit (E= evergreen, D= deciduous) was added to the traits used for calculating FDis and CWM. FDis and CWM were calculated in R (*R Core Team (2022),* package = "*dbFD*") (Laliberté et al., 2014).

Mixed models were evaluated using the "lmer_alt" function in the "LME4" package in R. We first evaluated a full model to assess Site, SR, Irrigation and CWM effects on Log transformed LAR (log-herb). The full model had the form:

log_herb ~ Site*SR*Irrigation*CWM +(1|Block_ID) + (1|Plot_ID)

All combinations of variables (including CWM values for the different quantitative traits) and possible interaction were tested to test the relevance of SR in predicting herbivory rates.

In a second step the same procedure was repeated substituting SR with FDis.

II.8 Results

II.8.1 The Influence of Species Richness and Irrigation Treatment

The most parsimonious model (AIC=618.8) with SR resulted to be:

log_herb ~ Site*SR*+CWM Mh +(1|Block_ID) + (1|Plot_ID).

The table below reports the p values for the variables of the model:

Table 3. *Regression Analysis Summary Showing the Effects of Site, Species Richness (SR), Community Weighted Mean of Maximum Height (CWM.Mh), and the Interaction between Site and SR on the Response Variable.*

	Estimate	St Error	df	t value	p
(Intercept)	111.449	0.20125	7.288.896	5.538	4.59E-07***
SiteOrphee	-108.821	0.15211	3.393.216	-7.154	2.88E-08***
SR	0.11783	0.036	9.057.065	3.273	0.00151**
CWM.Mh	0.05898	0.00827	5.820.041	7.132	1.73E-09***
SiteOrphee:SR	-0.13574	0.04396	8.805.101	-3.088	0.00269**

The analysis reveals significant findings: 'SiteOrphee' negatively affects the outcome, 'SR' and 'CWM.Mh' positively influence it, and the 'SiteOrphee:SR' interaction suggests a modifying effect. All factors are statistically significant.

A thorough evaluation of the statistical model was conducted to ensure its robustness and reliability (Figure 9). This comprehensive model diagnostics covered a series of checks and assessments as detailed below:

The Posterior Predictive Check revealed that the observed data closely align with the model-predicted data, indicating good model fit. The Linearity assessment showed a flat and horizontal reference line in the residuals plot, suggesting a linear relationship between predictors and the response variable is appropriate. The Homogeneity of Variance was confirmed by the random distribution of residuals across fitted values, with no apparent pattern, satisfying the assumption of constant variance.

Influential Observations were within acceptable limits, as indicated by the absence of data points outside the contour lines in the leverage plot, which means no single observation unduly influences the model's predictions. The Collinearity evaluation indicated low to moderate variance inflation

factors for most predictors, with only a few reaching higher levels, suggesting some collinearity that may require further investigation.

The Normality of Residuals was supported by a Q-Q plot where points closely followed the theoretical line, pointing to well-behaved residuals that fit the normal distribution. Lastly, the Normality of Random Effects for both Plot_ID and Block_ID was also affirmed by their respective Q-Q plots, showing that the random effects are normally distributed, which is essential for the validity of the random effects model used.
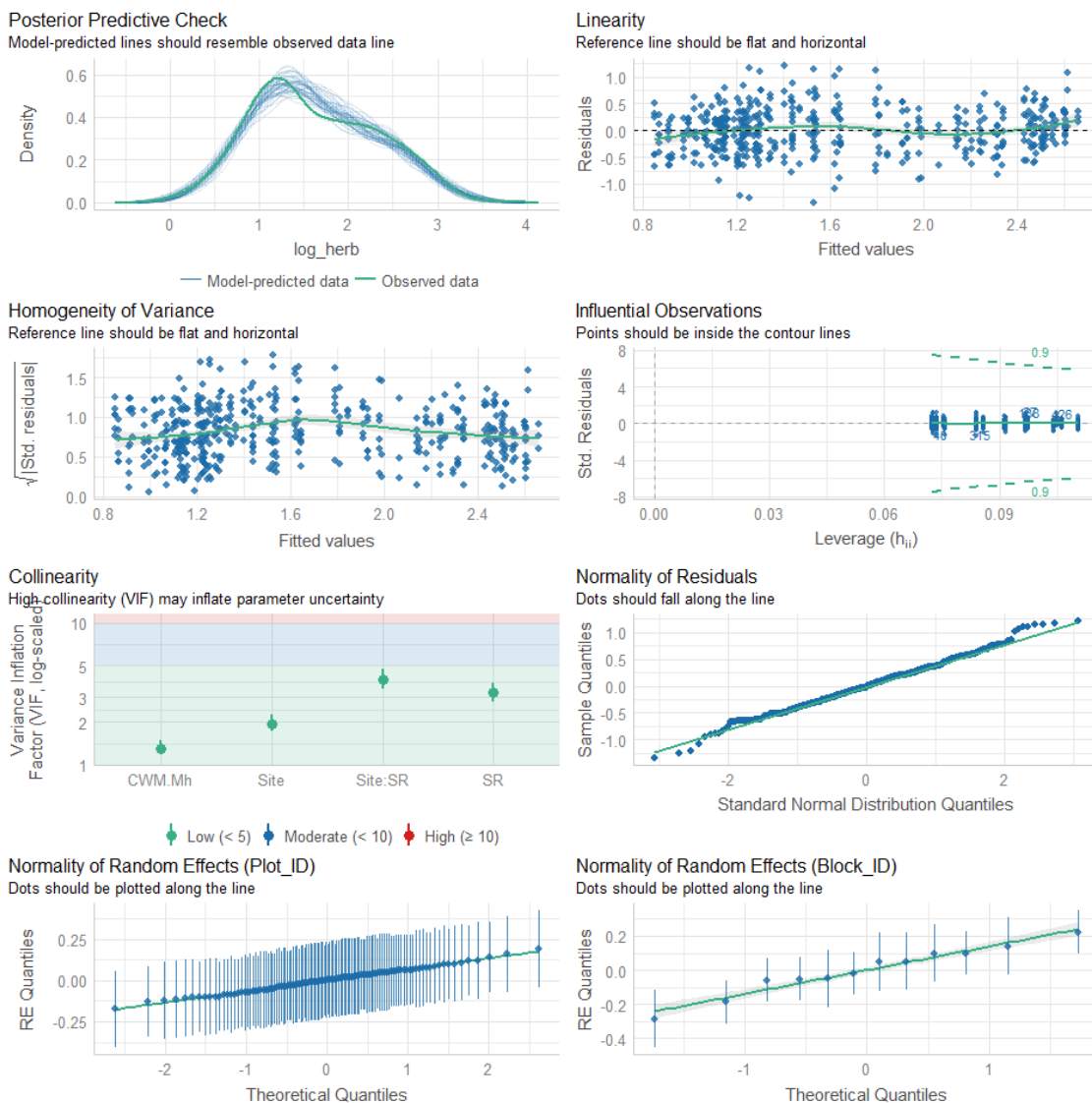
Figure 9. *Comprehensive Model Diagnostics including Posterior Predictive Check, Assessment of Linearity and Variance Homogeneity, Detection of Influential Data Points, Evaluation of Predictor Collinearity, and Examination of Residual and Random Effect Normality.*

The irrigation treatment, when included in the models was never significant (p>0.6).
Inclusion of CWM for SLA, WD or SDW entailed the loss of significance for SR and for the site*SR interaction.

Herbivory increased with CWM Mh in both sites. SR had no effect on herbivory in ORPHEE (slope not significantly different from zero) while herbivory increases with SR in IDENT-MACOMER (Figure 10).
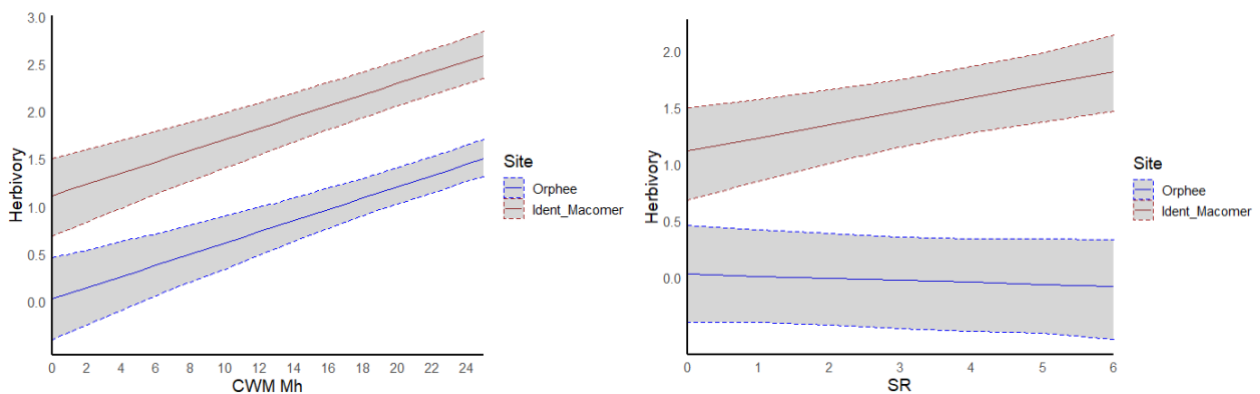
Figure 10. *Relationships between Herbivory and both CWM Mh and SR in IDENT-MACOMEER and ORPHEE sites.*

In our study, we found that herbivory damage on *Quercus ilex* L. leaves in the ORPHEE site was consistently lower than in the IDENT-MACOMER site, regardless of the level of species richness, including monoculture, two-species, three-species, and four-species conditions. Additionally, we observed that for water availability treatment (control and irrigated) did not have a significant effect on herbivory damage percentages in either site.

The most parsimonious model with FDis had the same form as the one with SR:
log_herb ~ Site*FDis*+CWM Mh +(1|Block_ID) + (1|Plot_ID).
The model had an AIC=602, 16.8 points less than the model using SR, and it is thus having a relevant higher performance.
Also, in this case the irrigation treatment had no effect on herbivory.
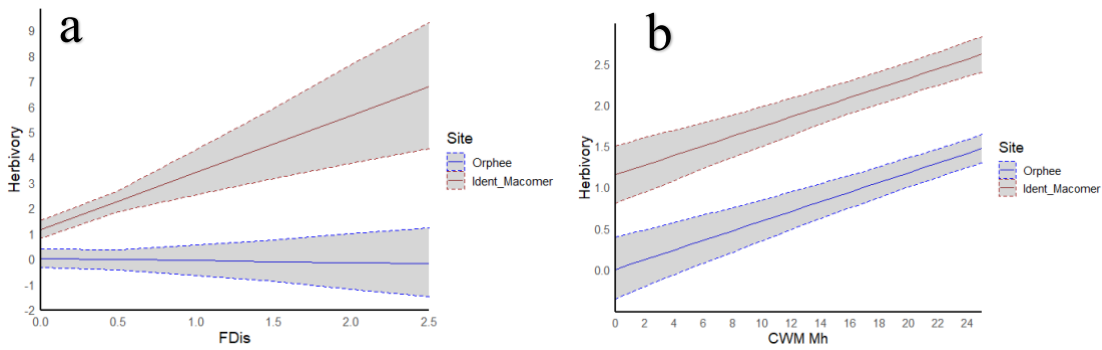
Figure 11. *Differential Impact of Functional Diversity (FDis) and Community Weighted Mean of Maximum Height (CWM Mh) on Herbivory* in IDENT-MACOMEER and ORPHEE *sites*.

The model with FDis confirms that the irrigation treatment had no effect on herbivory and confirms the relevance of CWM Mh with herbivory increasing in stands that contain species that tend to out compete their neighbors for light. Instead herbivory does not change in ORPHEE with FDis or it decreases at most supporting the dilution hypothesis. Instead it increases in IDENT_MACOMER

Figure 11a represents the relationship between FDis and herbivory rates in the 2 distinct ecological sites: ORPHEE and IDENT_MACOMER. A positive linear trend is evident for both sites, with increasing functional dispersion correlating with higher herbivory rates. The confidence interval for ORPHEE is significantly wider than that for IDENT_MACOMER, suggesting greater variability or less certainty in the herbivory measurements at ORPHEE. Moreover, the slope of the relationship at ORPHEE is steeper, indicating a more pronounced increase in herbivory with functional dispersion compared to IDENT_MACOMER. This may imply that trait diversity at ORPHEE has a stronger influence on herbivore feeding patterns, or that the site is subject to additional ecological factors not present at IDENT_MACOMER.

Figure 11b explores the influence of CWM Mh on herbivory at the same 2 ecological sites. Both sites exhibit a direct relationship between CWM Mh and herbivory, but the rate of increase is less steep compared to the relationship with functional dispersion. Notably, the confidence intervals for both sites are narrower than those observed in the functional dispersion analysis, suggesting more precise estimations of herbivory rates as a function of mean community height. The IDENT_MACOMER site shows a more consistent pattern, with less variation in herbivory at varying heights. In contrast, the broader confidence interval for ORPHEE may indicate a more complex interaction between plant height and herbivory, possibly due to differing herbivore preferences or other site-specific ecological dynamics.

56

Table 4. *Fixed Effects Estimates for Predictors of Herbivory. The table displays the parameter estimates, standard errors, degrees of freedom (df), t-values, and associated p-values for each fixed effect in the model. Asterisks denote the level of statistical significance, with three asterisks indicating p < 0.001.*

Fixed effects	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	115.124	0.17475	711.609	6.588	6.57e-09 ***
SiteOrphee	-11.461	0.13501	2.265.042	-8.489	1.73e-08 ***
FDis	224.866	0.52693	8.330.436	4.267	5.20e-05 ***
CWM.Mh	0.05855	0.00787	6.113.988	7.439	4.01e-10 ***
SiteOrphee:FDis	-23.228	0.59546	8.006.001	-3.901	0.000198 ***

The intercept represents the baseline level of herbivory when all predictors are held at zero. The significant negative estimate for SiteOrphee suggests that herbivory is lower at this site compared to the reference site by an average of 1.14610 units. FDis, which represents the functional dispersion, shows a positive association with herbivory, indicating that as functional dispersion increases, so does herbivory. CWM.Mh, which represents the community-weighted mean height, also shows a positive but much smaller effect. Interestingly, the interaction term SiteOrphee:FDis has a large negative coefficient, suggesting that the effect of functional dispersion on herbivory is different at the SiteOrphee compared to IDENT-MACOMER. The statistical significance of all these predictors is supported by their p-values, which are well below the 0.001 threshold, indicating a strong likelihood that these effects are not due to random chance.

II.8.2 Impact of Pine Tree composition
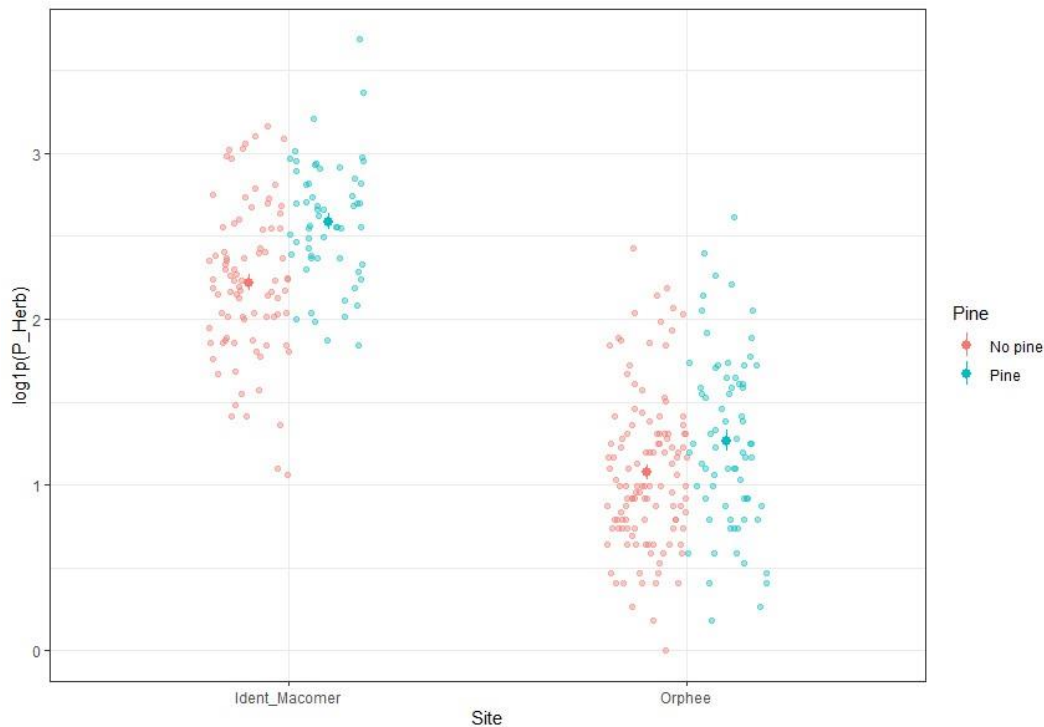
Figure 12. *Comparing herbivory assessment in both sites (IDENT-MACOMER and ORPHEE) with and without Pine trees*

The presence of pine trees within the tree composition demonstrated a consistent and pronounced influence on the herbivory assessment of *Q. ilex* in both the IDENT-MACOMER and ORPHEE sites. We consistently observed a substantial elevation in the levels of insect-induced damage on *Q. ilex* leaves when pine trees were present in mixed forest stands. Remarkably, this effect extended across various water availability treatments, encompassing both control and irrigated conditions.

More specifically, *Q. ilex* trees situated within stands containing pine trees exhibited significantly higher levels of insect herbivory damage when compared to trees in stands without pine trees. These findings underscore the substantial impact that the presence of pine trees exerts on the herbivory dynamics of *Q. ilex*, regardless of the specific water availability treatment in place.

Table 5. *Fixed Effects Estimates for the Impact of Site (ORPHEE), Pine Trees (Pine), and Irrigation on Quercus ilex L. Herbivory. The table presents estimates, standard errors, degrees of freedom, t-values, and p-values. Significance levels are denoted by asterisks (*p < 0.05, **p < 0.01, ***p < 0.001).*

Fixed effects	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	21.341	0.1358	150.941	15.71	9.19E-11***
Site	-0.8222	0.178	112.219	-4.619	0.000704***
Pine	0.43	0.1382	1.267.331	3.112	0.002296**
Irrigation	0.1531	0.1903	145.771	0.805	0.433953
Site:Pine	-0.5105	0.1763	1.240.392	-2.896	0.004471**
Site:Irrigation	-0.1986	0.2504	109.913	-0.793	0.444449
Pine:Irrigation	-0.1192	0.1962	1.274.675	-0.608	0.54446
Site:Pine:Irrigation	0.2366	0.2499	1.244.839	0.947	0.34554

The intercept, which represents the baseline condition, had an estimated value of 2.1341 with a standard error of 0.1358 and a highly significant t-value of 15.71 ($p < 0.001$). The variable 'Site' had an estimated coefficient of -0.8222, indicating a negative effect, and it was statistically significant with a t-value of -4.619 ($p = 0.000704$). 'Pine' exhibited a positive effect with an estimated coefficient of 0.43 ($p = 0.002296$), while 'Irrigation' showed a non-significant effect with a coefficient of 0.1531 ($p = 0.433953$). Interaction terms, such as 'Site:Pine', 'Site:Irrigation', 'Pine:Irrigation,' and 'Site:Pine:Irrigation' were also included in the analysis and displayed varying estimated coefficients and levels of significance. These results provide valuable insights into the relationships between the factors under investigation.

II.9 Discussion

The findings reveal a positive correlation between herbivory and the community-weighted mean height, hereafter referred to as the maximum potential height. It is imperative to distinguish between this trait and the realized height; the former indicates the species' inherent capacity to attain significant stature as a competitive strategy, predominantly for light acquisition, while the latter represents the actual measured height, which we did not assess in this study.

The maximum potential height, a trait commonly associated with rapid relative growth, is characteristic of species that exhibit pioneer dynamics, such as pines, which are known for their swift growth rates. However, this trait is not exclusive to pines, as it is also observed in other species like birch, indicating a broader adaptive strategy beyond a single taxonomic group.

This relationship suggests an ecological interplay where species with a greater potential height may modify the microclimate, corroborating the observations made regarding the 'pine effect' as seen. Notably, the inclusion of pine as an independent factor in our statistical models did not yield significant results, signifying that the observed herbivory patterns cannot be solely attributed to the presence of pines. This nuance underscores the complexity of the factors influencing herbivory beyond the scope of a single genus.

Furthermore, the functional dispersion of the community, as reflected by traits other than seed dry weight, seems to encapsulate aspects of the trees' architecture. Factors such as leaf phenology (evergreen vs. deciduous), leaf type (needle leaves), specific leaf area, and wood density contribute to this architectural diversity. Our interpretation posits that increased structural diversity, as denoted by functional dispersion, fosters higher levels of herbivory. This may be due to the creation of more intricate microhabitats, offering a plethora of secure niches for herbivores.

Considering these findings, it is crucial to acknowledge the structural complexity within these ecological communities as a potential driver for the diversity and abundance of herbivorous species. The intricate interplay between structural diversity and herbivory underscores the need for a multifaceted approach to understanding ecosystem dynamics.

Castagneyrol et al. (2018) demonstrated that tree species composition and irrigation treatments independently and interactively influence herbivore performance traits, such as growth and food

processing. Their study sheds light on the intricate interplay between tree diversity, abiotic conditions, and insect herbivory, underscoring the need to incorporate these factors into forest management and conservation strategies.

Our observations align closely with recent scholarly work, highlighting the significant impact of tree species diversity on reducing herbivory in forest ecosystems. Notably, studies have demonstrated that increased species richness can significantly support tree resistance against insect pests, thereby underscoring the protective benefits conferred by biodiversity (Jactel et al., 2021). This protective effect appears particularly pronounced for specialist herbivores in mixed stands comprising phylogenetically distant tree species, suggesting that not just the richness but the composition of tree species plays a crucial role in forest resilience to herbivory (Jactel et al., 2007). These insights not only validate our hypotheses regarding the influence of species diversity on herbivory rates but also highlight the complicated relationship between tree diversity and ecosystem health, thereby offering valuable perspectives for sustainable forest management and conservation strategies.

II.10   Conclusion

This study investigates the multifaceted relationship between tree species diversity, water availability, tree height differences, leaf palatability, and microenvironmental conditions in *Quercus ilex* L. stands dominated by pine trees. Notably, it is conceivable that *Q. ilex* trees cohabiting with pine trees may offer more palatable leaves, as shaded leaves are typically thinner, softer, and richer in water content, potentially attracting herbivorous insects (Coley & Barone, 1996; Fajardo et al., 2013). Additionally, the understory beneath the shade of pine trees may provide a more favorable environment for insects, characterized by reduced temperature fluctuations, increased air humidity, and reduced radiation levels. These conditions could create a microenvironment conducive to higher insect abundance and activity (Chen et al., 2018; Del Toro et al., 2013).

This notable outcome indicates the possibility of interactions or facilitation between pine trees and herbivorous insects, thus calling for further investigation into the underlying mechanisms. Furthermore, the outcomes underscore the crucial role of considering the composition of tree species when assessing herbivory impacts within mixed forest ecosystems. This consideration holds significant implications for ecosystem management and conservation strategies.

The potential influences driving this phenomenon may encompass species interactions, competitive dynamics, and the intricate chemical ecology of both tree species. Furthermore, environmental factors, notably microclimates and soil conditions, may play pivotal roles in shaping these herbivory patterns. Comprehensive understanding of these multifaceted dynamics is indispensable for clarifying the observed variations in herbivory and for developing effective strategies for the management and preservation of mixed forests. Further exploration is warranted to delve into the ecological and chemical mechanisms governing these interactions, thereby enhancing our understanding of this intricate ecological interplay.

References

Barbero, M. R. P. F., Loisel, R., and Quézel, P. (1992). Biogeography, ecology and history of Mediterranean Quercus ilex ecosystems. Quercus ilex ecosystems: function, dynamics and management, 19-34.

Castagneyrol, B., Giffard, B., Péré, C., and Jactel, H. (2014). Plant apparency, an overlooked driver of associational resistance to insect herbivory. Journal of Ecology, 102(2), 418-429.

Castagneyrol, B., Régolini, M., and Jactel, H. (2014). Tree species composition rather than diversity triggers associational resistance to the pine processionary moth. Basic Appl. Ecol. 15, 516–523. Castro, J., Zamora, R., 2004.

Castagneyrol, B., Moreira, X. & Jactel, H. Drought and plant neighbourhood interactively determine herbivore consumption and performance. Sci Rep 8, 5930 (2018). https://doi.org/10.1038/s41598-018-24299-x

De Rigo, D. and Caudullo, G. (2016). Quercus ilex in Europe: distribution, habitat, usage and threats. In: De Rigo, D. et al. (eds), European Atlas of forest tree species. Publications Office of the European Union, pp. 152–153.

Diaz, S., Hector, A., & Wardle, D. A. (2007). Biodiversity in forest carbon sequestration initiatives: not just a side benefit. Current Opinion in Environmental Sustainability, 1(1), 55-60.

Ente Autonomo Flumendosa, (1998). Studio sull'Idrologia Superficiale della Sardegna.Regione Autonoma della Sardegna, Assessorato della Programmazione, Bilancio ed Assetto del Territorio, Cagliari.

Ghirardo, A., Heller, W., Fladung, M., Schnitzler, J.-P. and Sschroedeer, H. (2012), Function of defensive volatiles in pedunculate oak (Quercus robur) is tricked by the moth Tortrix viridana. Plant, Cell & Environment, 35: 2192-2207. https://doi.org/10.1111/j.1365-3040.2012.02545.x

Hernández-Agüero, J. A., Ruiz-Tapiador, I., & Cayuela, L. (2022). What feeds on Quercus ilex? A biogeographical approach to studying trophic interactions in a Mediterranean keystone species. Diversity and Distributions, 28, 4–24. https://doi.org/10.1111/ddi.13413

Holzinger, R., Sandoval-Soto, L., Rottenberger, S., Crutzen, P. J., and Kesselmeier, J. (2000), Emissions of volatile organic compounds from Quercus ilex measured by Proton Transfer Reaction Mass Spectrometry under different environmental conditions, J. Geophys. Res., 105(D16), 20573–20579, doi:10.1029/2000JD900296.

Jactel H, Brockerhoff EG. Tree diversity reduces herbivory by forest insects. Ecol Lett. (2007) Sep;10(9):835-48. doi: 10.1111/j.1461-0248.2007.01073.x. PMID: 17663717.

Jactel, H., Bauhus, J., Boberg, J., Bonal, D., Castagneyrol, B., García-Valdés, R. and Castagneyrol, B. (2019). Tree diversity drives forest stand resistance to natural enemies. Current Biology, 29(13), 4429-4433.

Jactel H, Moreira X, Castagneyrol B. Tree Diversity and Forest Resistance to Insect Pests: Patterns, Mechanisms, and Prospects. Annu Rev Entomol. (2021) Jan 7;66:277-296. doi: 10.1146/annurev-ento-041720-075234. Epub 2020 Sep 9. PMID: 32903046.

Letourneau, D. K., Jedlicka, J. A., Bothwell, S. G., and Moreno, C. R. (2011). Effects of natural enemy biodiversity on the suppression of arthropod herbivores in terrestrial ecosystems. Annual Review of Ecology, Evolution, and Systematics, 42, 573-592.

Moreira, X., Castagneyrol, B., de la Mata, R., Fyllas, N. M., Galmán, A., García-Verdugo, C., Larrinaga, A. R., & Abdala-Roberts, L. (2019). Effects of insularity on insect leaf herbivory and chemical defences in a Mediterranean oak species. *Journal of Biogeography*, *46*(6), 1226–1233. https://doi.org/https://doi.org/10.1111/jbi.13589

Moreira, X., Abdala-Roberts, L., Galmán, A., Bartlow, A. W., y Teran, J. C., Carrari, E., Covelo, F., de la Fuente, M., Ferrenberg, S., Fyllas, N. M., Hoshika, Y., Lee, S. R., Marquis, R. J., Nakamura, M., Nell, C. S., Pesendorfer, M. B., Steele, M. A., Vázquez-González, C., Zhang, S., & Rasmann, S. (2020). Ontogenetic consistency in oak defence syndromes. *Journal of Ecology*, *108*(5), 1822–1834. https://doi.org/https://doi.org/10.1111/1365-2745.13376

Ruiz-Carbayo, H., Bonal, R., Espelta, J. M., Hernández, M., & Pino, J. (2017). Community assembly in time and space: the case of Lepidoptera in a Quercus ilex savannah-like landscape. *Insect Conservation and Diversity*, *10*(1), 21–31. https://doi.org/https://doi.org/10.1111/icad.12184

Rodà F, Retana J, Gracia CA, Bellot J. (1999a). Ecology ofMediterranean evergreen oak forest. Springer, Berlin.

Root, R. B. (1973). Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards (Brassica oleracea). Ecological Monographs, 43(1), 95-124.

Schuldt, A., Baruffol, M., Böhnke, M., Bruelheide, H., Härdtle, W., Lang, A. C., and Assmann, T. (2010). Tree diversity promotes insect herbivory in subtropical forests of south-east China. Journal of Ecology, 98(4), 917-926.

Southwood TRE, Wint GRW, Kennedy CEJ, Greenwood SR. (2005). The composition of the arthropod fauna of the canopies of some species of oak (Quercus). Eur J Entomol 102:65–72. https://doi.org/10.14411/eje.2005.009

Tariq, M., Wright, D. J., Bruce, T. J., and Staley, J. T. (2013). Drought and root herbivory interact to alter the response of above-ground parasitoids to aphid infested plants and associated plant volatile signals. PloS one, 8(7), e69013.

Vila, M., Maron, J. L., Marco, L., & Díaz, M. (2007). Evidence for the enemy release hypothesis in Hypericum perforatum. Oecologia, 150(3), 472-480.

III. Chapter 3 The Stress Gradient Hypothesis in Mediterranean tree communities

III.1 Abstract

The Stress Gradient Hypothesis (SGH) posits that species interactions are influenced by resource availability, with facilitative interactions increasing under high stress conditions and competitive interactions dominating under low stress conditions. In this study, we tested the SGH in Mediterranean tree communities as it has not been tested before.

A nursery experiment was established in Macomer, Italy, using 12 Mediterranean tree species. The experiment consisted of monocultures and mixed plots with varying species richness and functional diversity. Stem diameters were measured annually and stem basal area was used as a proxy for biomass. Results indicate that functional diversity and species richness have significant effects on the net, positive, and competitive effects of biodiversity on ecosystem functioning. However, there were no significant interactions between species richness or functional diversity and irrigation treatment. Irrigated blocks showed higher net and competitive effects compared to rain-fed blocks. Similarly, to what was found in the IDENT-SSM site (Canada) with boreal species, the SGH is not confirmed and results show that the net effects and complementary effects of biodiversity on Ecosystem functions are greater in conditions of higher water availability. These findings provide insights into the complexity of species interactions and their influence on ecosystem functioning in Mediterranean tree communities suggesting caution in the use of diverse forests as a Nature Based Solution. Furthermore, these findings suggesting that the expected increase in aridity in the Mediterranean region could have a relatively higher impact on mixed tree communities compared to monocultures. Overall, this research contributes to our understanding of managing and restoring ecosystems in the context of climate change and biodiversity loss.

Mediterranean forests; drought stress effects; biodiversity effects; ecosystem functioning; water availability; species interactions

III.2 Introduction

Since the beginning of modern ecology, scientists have been intrigued by the enigmatic intricacies of species interactions and their profound influences on ecosystem processes. Pioneering ecologists such as Charles Elton and Aldo Leopold highlighted the foundational role of biotic interactions in maintaining the equilibrium and resilience of ecosystems (Elton, 1927; Leopold, 1949). This traditional curiosity has now evolved into an imperative, primarily in the face of anthropogenic challenges. With climate change imperiling global ecosystems and biodiversity dwindling at an alarming rate, understanding these biotic interactions has transcended academic interest. It's now a clarion call for urgent action, especially as the international community grapples with daunting targets set by accords like the Paris Agreement (Rogelj et al., 2016) and the Convention on Biological Diversity (CBD, 2020).

Understanding how species interactions ultimately affect ecosystem process has been a long-standing challenge in ecology (Balvanera et al., 2006; Cardinale et al., 2012). This research field is today becoming more relevant as we are in need to manage, conserve, and restore ecosystems in ways that will significantly contribute to reaching global goals for ameliorating climate change and biodiversity loss. The study of species interactions can contribute to developing a predictive understanding of ecosystem response to persistent environmental impacts (Tylianakis et al. 2008; Harmon et al. 2009). Namely, the ability to pinpoint what mixture of species may yield higher productivity and resilience, as well as the potential response to environmental conditions, has a major role in improving management strategies in the current global change context.

Central to this discourse on ecosystem health and resilience is biodiversity. Over the years, a multitude of studies has elucidated the invaluable role of biodiversity in underpinning ecosystem services – the very services that human survival hinges upon. From the prairies of North America to the coral reefs of the Indo-Pacific, the dictum appears consistent: higher biodiversity often translates to improved ecosystem functionality, be it in terms of productivity, nutrient cycling, or resistance to perturbations (Balvanera et al., 2006). In the realm of forestry, this understanding has profound implications. Forests, with their immense carbon sequestration potential, stand at the forefront of climate mitigation strategies. Studies from the Amazon to Borneo have revealed that tree species richness can amplify forest productivity, enhancing their carbon capture capacity (Poorter et al., 2015).

Overall, observational and experimental studies have shown positive effects of biodiversity on ecosystem functioning (BEF) for a multitude of system types (Cardinale et al. 2011; hooper et al. 2012) and more lately also in forests and plantations (Xu et al. 2020). Although positive in general, this relationship is not always positive (e.g., Vilà et al. 2003; Jiang et al. 2009) thus limiting the predictability of the outcomes in mixing species and consequently the application in real world context as for example in Nature Based Solutions. The Net Effect (NE) of biodiversity on Ecosystem Functions can be seen as the balance between positive and negative interactions, but this balance depends on many factors such as species traits, trait similarity and size of neighboring individuals, functional redundancy, age of the stand, available resources, climate, biotic agents and disturbances (Walker 1992; Naeem 1998; Forrester & Bauhus, 2016; He et al., 2013). The NE of positive BEF relationships are typically explained by the combination of two non-mutually exclusive mechanisms: complementarity and selection effects (Loreau & Hector, 2001). Complementarity effects are related to reduced competition due to greater heterogeneity of traits that improve productivity, including niche partitioning, facilitation and positive feedback on resource supply (Forrester & Bauhus, 2016; Hisano et al., 2018; Kelty, 2006). On the other hand, selection effects are due to highly competitive species which reach disproportionately large growth rates and as result drive ecosystem functioning (Roscher et al. 2012). It has been proved that these mechanisms frequently work together in a variety of ecosystems, and the complementarity effects often explain more proportion of the variance (Reich et al. 2012; Cardinale et al. 2011).

To deepen our understanding of these fundamental ecological concepts, BEF research in tree communities has grown considerably in the past two decades with a growing number of experiments manipulating species communities. However, most of the BEF experiments have been classically conducted on temperate or subtropical systems (e.g. Maxwell et al., 2022[LM1]; Hajek et al., 2022[LM2]; Belluau et al., 2021), while research on seasonally dry Mediterranean ecosystems is both a relatively recent development and uncommon. Climate has been shown to strongly affect the BEF relationships of plant communities but results often vary by region (Ammer, 2019; Ratcliffe et al., 2017). Plant communities located in Mediterranean areas are exposed to a few climatic characteristics with ecological implications that justify the need of considering BEF relationships in these ecosystems with special focus. On the one hand, temperature rises due to climate change are more pronounced in the Mediterranean basin than in other areas, as annual mean temperature has increased by 1.5 °C compared to a 1.1 °C global increase (Ali et al., 2022). On the other hand, the length of the dry season ranges between 3 and 5 months, with soil water potentials reaching extremely low values during this period (Ali et al., 2022).

68

Moreover, as aridity increases so does functional convergence (Costa-Saura et al., 2017 Ali et al., 2022), which implies that a high functional diversity that could lead to complementarity or positive NE may not be reachable at Mediterranean areas due to the extremely dry seasonal climate.

Experimental studies have proved that high-diversity plant communities tend to be more productive than monocultures, thus providing larger shade, cooler temperatures, increased likelihood of vicinity of deep-rooted species, and raised air humidity and top-soil moisture at the community level (Wright et al. 2014, Steinauer et al. 2015, Cowles et al. 2016). In line with these findings, the Stress Gradient Hypothesis (SGH) predicts that positive plant interaction effects (i.e., facilitation) on productivity are of greater relative importance in habitats subject to abiotic stresses, while competition and other negative plant interactions are the main driver of productivity in more protected ecosystems (Bertness & Callaway 1994). Interestingly, the selection effect tends to be comparatively more relevant in environments where abiotic conditions are not stressors, whereas the complementarity effect is generally the dominant mechanism in stressed environments (Paquette & Messier, 2011; Ratcliffe et al., 2017; Wang et al., 2013). Although this hypothesis has been widely supported in the literature (see Callaway 2007), the generality of the SGH to a variety of ecosystems is currently subject of debate as experimental proof has both confirmed and refuted it (He et al., 2013; Maestre et al., 2009; Brooker 2006). An illustrative example can be found in arid and semi-arid ecosystems where mixed evidence for this hypothesis has been reported in studies on water or rainfall gradients (Tielborger & Kadmon 2000; Maestre & Cortina 2004; Armas & Pugnaire 2005; Holzapfel et al. 2006). These incongruent results have been attributed to the high diversity of abiotic stresses (e.g. physical, resource, grazing), methodological differences (Goldberg et al. 1999; Gross et al. 2010), plant species characteristics (e.g. life histories, functional traits Brooker et al., 2008; He et al., 2013), experimental vs descriptive studies (Maestre et al. 2005; Michalet 2006) and ecosystem types (Lortie & Callaway 2006; Maestre et al. 2009), which constrain the development of a widely applicable conceptual model of how species interactions shift with increasing stress.

Yet, the influence of biodiversity is full of nuances Its relationship with ecosystem function, though generally positive, exhibits variability underpinned by a matrix of ecological, evolutionary, and environmental determinants. The net effect (NE) of biodiversity is a culmination of both synergistic (facilitative) and antagonistic (competitive) interactions among species. But this delicate equilibrium is modulated by myriad factors: from the evolutionary lineage and functional traits of species to externalities such as disturbances and resource availability (Cadotte et al., 2011). This unpredictability sometimes hampers conservation endeavors, particularly when we attempt to harness

69

biodiversity for tangible outcomes like in Nature-Based Solutions (NBS). The challenges are manifold: while some multi-species interventions yield anticipated outcomes, others confront unforeseen ecological dynamics, underscoring the imperative for a deeper understanding (Suding et al., 2015).

Amid this backdrop, research on biodiversity and ecosystem function (BEF) in tree communities has burgeoned, but with a conspicuous lacuna: the Mediterranean ecosystems remain relatively uncharted. These ecosystems, characterized by their distinct bioclimatic regimes, present a unique conundrum. On one hand, they are grappling with exacerbated climate change impacts, with temperature anomalies starkly outpacing global averages (Luterbacher et al., 2012). On the other, they are arenas of intense ecological drama during prolonged dry seasons, when soil desiccation challenges the very survival of tree communities. Such extreme conditions engender functional convergence among tree species, making these ecosystems increasingly homogenous, thereby potentially compromising the very benefits of biodiversity (Gazol et al., 2018).

Although species richness has been the most commonly used measure in BEF research, there is growing evidence that it may not be the most efficient predictor of ecosystem functioning. Functional traits are fundamental to a mechanistic understanding of BEF (Reiss et al. 2009), as these traits link species to their roles in the ecosystem, impacting fitness through their effects on growth, reproduction, and survival (Violle et al. 2007). Therefore, functional diversity (the diversity of traits in a community) emerges as a powerful metric to quantify BEF mechanisms (Díaz & Cabido 2001; Mokany et al. 2008; Gravel et al. 2011). However, both species richness and functional diversity are undeniably linked in the natural world, and their covariance challenges our ability to disentangle their respective effects on BEF (Naeem 2002; Paquette and Messier 2011). To delve into the potential role of functional traits on plant interactions, numerous traits have been explored in these experiments and they have been increasingly found to mediate the outcome of plant interactions (Sthultz et al. 2007; Gomez-Aparicio 2009; He et al. 2012). In this context, it has been proposed that functional traits may affect how species interactions change along stress gradients (Lortie & Callaway 2006; Castanho et al. 2012).

The Stress Gradient Hypothesis (SGH) provides an intriguing lens to assess these dynamics. Rooted in the idea that species interactions evolve along environmental stress gradients, SGH posits that facilitative interactions gain prominence under high stress, whereas competition reigns supreme under

resource abundance (Bertness and Callaway, 1994). However, the empirical journey of SGH, though rich, is also riddled with contradictions. While some ecosystems validate its tenets, others challenge its universality. This inconsistency accentuates the need for targeted investigations, especially in under-studied regions like the Mediterranean. Our experiment, set against this backdrop, aims to unravel the intricacies of the SGH within the unique context of Mediterranean tree communities.

In this study, we performed a common garden experiment manipulating the species composition of Mediterranean tree communities and exposed them to high- and low-water availability to test for the SGH in these environments. In this diversity experiment, trees are planted using high-density tree communities in monocultures and mixtures following a well-balanced design (Tobner et al., 2014). To determine the effects of functional diversity on productivity disregarding the effect of species richness, species mixtures were selected by using 12 Mediterranean tree species grouped in identical species richness which vary in functional diversity (Tobner et al. 2014). Interestingly, this study is one of the two unique tree diversity experiments located in dry (Mediterranean) climate. Here, we present the first results of this experiment, 9 years after its establishment. Specifically, we tested the following hypotheses:

i- Biodiversity effects on NE, CE, and SE are stronger at lower water availability
ii- FD and CWM are both strong predictors of NE, CE, and SE with all three increasing with FD and the effect of FD being stronger at lower water availability

The table below provides concise definitions and key ideas for Complementary Effect (CE), Net Diversity Effect (NE), and Selection Effect (SE) within the context of biodiversity and ecosystem functioning. These concepts clarify the involved relationships between species diversity and the dynamic processes shaping ecosystem resilience, productivity, and adaptation (Loreau and Hector, 2001; Tilman et al., 1997; Cardinale et al., 2006).

Table 1. *Concepts of Net Diversity Effect (NE), Complementary Effect (CE) and Selection Effect (SE) in Biodiversity and Ecosystem Functioning.*

Concept	Definition	Key Idea
Net Diversity Effect (NE)	Overall impact of biodiversity, considering both positive (ecosystem services) and negative (competition) influences.	Net Diversity Effect assesses the comprehensive impact of biodiversity on ecosystem functioning, weighing both benefits and challenges.
Complementary Effect (CE)	Positive synergy when diverse species enhance each other, resulting in a more resilient and productive ecosystem.	Biodiverse ecosystems experience a Complementary Effect, where different species contribute to overall ecosystem health and function synergistically.
Selection Effect (SE)	Process where certain species are naturally selected based on environmental conditions, leading to a diverse and adaptive community.	Ecosystems undergo a Selection Effect as species are naturally chosen based on their compatibility with prevailing environmental conditions, contributing to biodiversity.

III.3 Materials and methods

III.3.1 Study site

IDENT-MACOMER is located within the "Saint Antonio - Sardinian Forest Authority" nursery in Macomer, Sardinia, Italy. It is an integral part of the International Diversity Experiment Network with Trees (IDENT) and sits at an elevation of 640 meters above sea level. The prevailing local climate falls under the classification of hot-summer Mediterranean (Köppen: Csa), characterized by its warm, arid summers, and mild winters accompanied by moderate precipitation.

Previously utilized as a tree nursery for regional reforestation endeavors until 2008, the area was subsequently left unmanaged, with sporadic removal of grasses. In 2015, a meteorological station adjacent to the experimental site was installed measuring precipitation, photosynthetic active radiation, wind speed and direction at 2m height, air temperature, and relative humidity at 0.2m, 1m, 2m, 4m height. All data is acquired at 5-minute intervals and stored as 30-minute averages.

Climatic data over the period 1981-2010 (Figure 1) was downloaded from the Regional Agency for Environmental Protection (ARPAS, https://arpas.maps.arcgis.com) for the station of Macomer, 5.2 km from the site. However, data from the meteorological station in the site for the years 2015-2021 differ substantially from the climatic means.
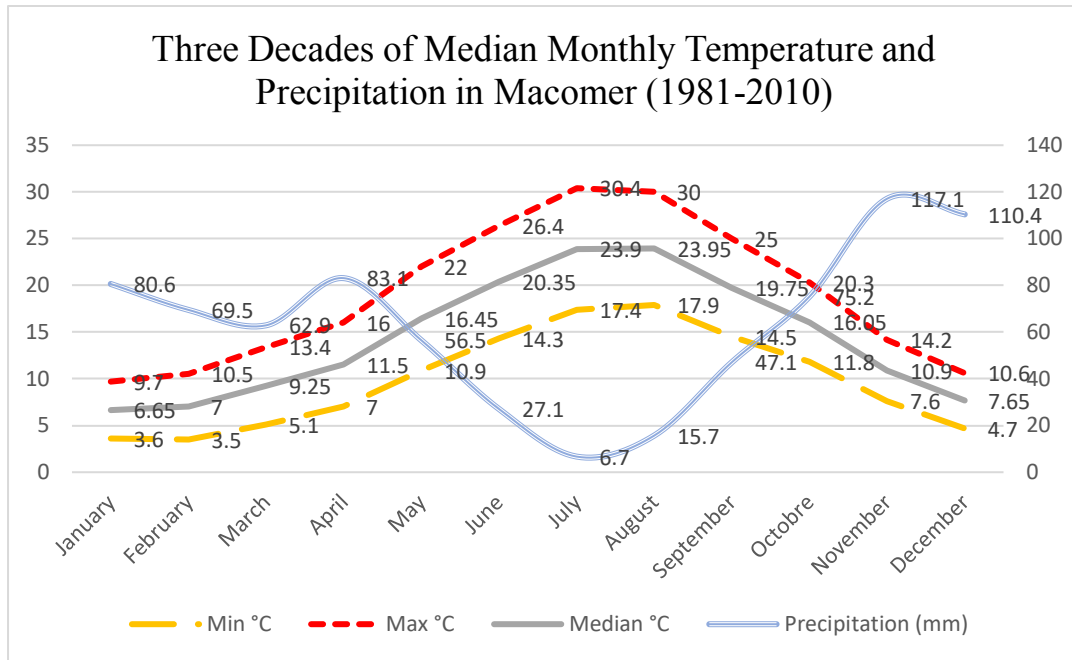
Figure 1. *Three Decades of Median Monthly Temperature and Precipitation in Macomer (1981-2010). This figure illustrates the variations in median monthly temperature and precipitation patterns over a thirty-year period, providing valuable insights into the climatic trends of the Macomer.*

From 2015 to 2022, monthly average temperatures span from 6.5 °C in January to 23.9 °C in August. Concurrently, monthly rainfall varies from 135 mm in December to a mere 7 mm in July, with an annual accumulation of approximately 905 mm.

The selection of species for this experiment was contingent on site-specific requirements and the availability of seedlings in the local nursery. In total, 12 native woody species were chosen (Table 2), encompassing both shrubs (three species) and trees (nine species).

Table 2. *Taxonomic classification, species code, common nomenclature, and leaf habit characteristics of studied tree species (IDEENT-M).*

Latin name	Species code	Common name	Leaf habit
Quercus ilex L.	QUIL	Holm Oak	Evergreen
Quercus suber L.	QUSU	Cork Oak	Evergreen
Quercus pubescens Willd.	QUPU	Downy Oak	Deciduous (Marcescent)
Pinus pinaster Ait.	PIPA	Maritime Pine	Evergreen
Pinus pinea L.	PIPE	Stone Pine	Evergreen
Pinus halepensis Mill.	PIHA	Aleppo Pine	Evergreen
Phillyrea latifolia L.	PHLA	Green Olive Tree	Evergreen
Acer monspessulanum L.	ACMO	Montpellier Maple	Deciduous
Pistacia lentiscus L.	PILE	Mastic Tree	Evergreen
Arbutus unedo L.	ARUN	Strawberry Tree	Evergreen
Fraxinus ornus L.	FROR	Manna Ash	Deciduous
Olea europea L.	OLEU	Olive Tree	Evergreen

III.3.2   Experimental design

The experimental layout incorporates a hierarchical arrangement of trees distributed across 308 plots and organized into seven distinct blocks (Figure 2). Each block encompasses 44 plots measuring 3.2 m by 3.2 m, and within each plot, 64 seedlings were planted at 40 cm intervals. The high density of the plots ensures the early closure of canopies and interactions among individuals. In each block, 12 plots were planted as a monoculture, 17 as combinations of two of the 12 species, 9 as combinations of four species and 6 as combinations of 6 species. The composition for each plot was designed to create a functional diversity (FD) gradient for each species richness level (SR= 1,2,4,6). FD was quantified by computing the Functional dispersion index (FDis) with the R package "*dbFD*" (Laliberté et al., 2014) based on the method by Laliberte and Legendre (2010). FDis quantifies the dissimilarity of species within a community. For the design of the experiment, species traits to compute FDis were retrieved from the literature and consisted in: SLA, Nmass, Wood Density, Max Stomatal Conductance (Gsmax), PNmax, leaf area, P50, Minimum water potential. The FDis used for the statistical analysis is instead based also on traits assessed on the planted individuals to achieve a higher accuracy. These were: LMA, Nmass and a new trait, the Huber value.

The distribution of species within each plot was randomized with some restrictions to avoid clusters: four individuals of the same species could not be arranged to form a square, ii) five individuals of the same species could not be arranged to form a cross, and iii) at least 2 of the 8 neighboring individuals had to be of a different species.

Furthermore, the relative abundance of species is the same in the center 4 by 4 individuals, in the 28 individuals composing the outer frame (boundary trees) and in the 20 individuals composing the frame between the outer frame and the center 4 by 4.

During the inaugural year (2014), all blocks received irrigation during the dry season because the first spring and summer of life of seedlings is the most critical stage for the establishment of Mediterranean woody species (Mendoza et al., 2009). In the following years, three selected blocks underwent irrigation with 20 mm of water administered every 15 days from June to September (+160mm in total), facilitated by a pipe system. In contrast, the remaining four blocks were designated as controls and did not receive irrigation.
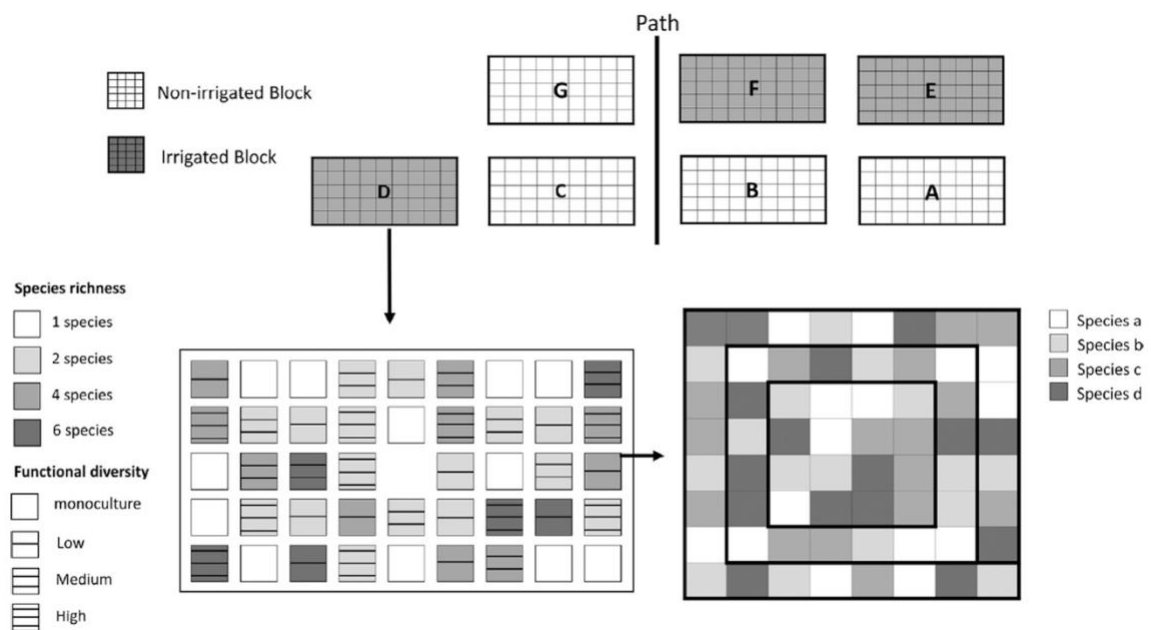
Figure 2. *Experimental design of IDENT-MACOMER. (Top) Site plan with three irrigated (grey) and four non-irrigated (white) blocks. Block A was not considered because trees were planted earlier (during extreme wet conditions), with clear impacts on the performance of all plants (Under left) disposition of 44 plots within a block (the center position is empty), including orthogonal gradients of species richness and functional diversity: (Under right) arrangement of 64 plants in a four-species plot with outer, middle and inner plot frame differentiated by bold lines.*

Dead individuals were replaced for the first 2 years with trees of the same species and age that were planted at the same time in spaces around the experimental blocks. Only one species displayed a high mortality, *Pistacia lentiscus* L., likely as a result of a combination of low temperatures (*P. lentiscus* is a thermophilus species) and small seedling size. Although not monitored, a substantial mortality occurred during weeding operations. The table below reports species mortality in the first two years as a percentage of the total individuals planted per species.

The table below provides a comprehensive mapping between tree community composition codes (code mix) and the corresponding species combinations. Each unique code mix is associated with a specific combination of tree species, offering a detailed reference for understanding the composition of diverse tree communities within the study.

Table 3. *Tree community composition codes and corresponding species combinations*

Code mix	Species combination	Code mix	Species combination
2n1a	ACMO/QUPU	2n3f	QUSU/PIHA
2n1b	ARUN/PILE	4n1a	QUIL/QUSU/OLEU/PHLA
2n1c	OLEU/PHLA	4n1b	ARUN/PILE/PIPA/PIHA
2n1d	PIPA/PIHA	4n1c	PIPE/FROR/ACMO/QUPU
2n1e	PIPE/FROR	4n2a	QUIL/OLEU/ARUN/PIPA
2n1f	QUIL/QUSU	4n2b	QUPU/FROR/PIHA/PILE
2n2a	QUIL/PHLA	4n2c	QUSU/PHLA/PIPE/ACMO
2n2b	PIHA/ACMO	4n3a	QUIL/PHLA/PIPA/FROR
2n2c	PIPA/FROR	4n3b	QUSU/ARUN/PIHA/ACMO
2n2d	PIPE/QUPU	4n3c	OLEU/PILE/PIPA/QUPU
2n2e	QUSU/ARUN	6n1a	QUIL/QUSU/OLEU/PHLA/ARUN/PILE
2n3a	ARUN/ACMO	6n1b	PIPA/PIHA/PIPE/FROR/ACMO/QUPU
2n3b	OLEU/PIPE	6n2a	QUIL/QUSU/ARUN/PILE/PIPE/FROR
2n3c	PHLA/FROR	6n2b	OLEU/PHLA/PIPA/PIHA/ACMO/QUPU
2n3d	PILE/QUPU	6n3a	QUIL/OLEU/ARUN/PIPA/PIPE/ACMO
2n3e	QUIL/PIPA	6n3b	QUSU/PHLA/PILE/PIHA/FROR/QUPU

Table 4. *Mortality rates (Numbers and Percentages) of studied tree species under irrigation and control conditions across multiple years.*

	2015		2016		2017		2018		2019		2020		2021		2022	
Species	Control	Irrigation	Control	Irrigation	Control	Irrigation	Control	Irrigation	Control	Irrigation	Control	Irrigation	Control	Irrigation	Control	Irrigation
Acer monspessulanum **L.**	4 (0,3)	2 (0,1)	12 (0,8)	1 (0,1)	18 (1,3)	1 (0,1)	20 (1,5)	3 (0,2)	47 (3,5)	42 (3,1)	90 (7,2)	110 (8,9)	96 (7,8)	111 (9)	99 (8,1)	123 (10,1)
Arbutus unedo **L.**	1 (0,1)	3 (0,2)	2 (0,1)	5 (0,3)	4 (0,3)	10 (0,8)	4 (0,3)	10 (0,7)	4 (0,3)	12 (0,8)	5 (0,4)	17 (1,2)	7 (0,5)	19 (1,3)	8 (0,6)	19 (1,3)
Fraxinus ornus **L.**	1 (0,1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	13 (1)	4 (0,3)	30 (2,2)	31 (2,2)	54 (4,1)	73 (5,6)	56 (4,3)	74 (5,6)	61 (4,7)	83 (6,4)
Olea europea **L.**	3 (0,2)	6 (0,5)	5 (0,4)	10 (0,8)	10 (0,8)	10 (0,9)	17 (1,5)	32 (2,7)	41 (3,6)	55 (4,8)	53 (4,7)	75 (6,7)	62 (5,6)	69 (6,2)	63 (5,8)	91 (8,3)
Phillyrea latifolia **L.**	3 (0,2)	4 (0,3)	2 (0,1)	1 (0,1)	4 (0,3)	1 (0,1)	6 (0,5)	5 (0,4)	9 (0,6)	3 (0,2)	11 (0,8)	8 (0,6)	13 (0,9)	8 (0,6)	15 (1,1)	20 (1,4)
Pinus halepensis **Mill.**	1 (0,1)	4 (0,3)	3 (0,2)	11 (0,8)	5 (0,4)	17 (1,3)	9 (0,7)	29 (2,1)	18 (1,3)	51 (3,7)	34 (2,6)	70 (5,3)	36 (2,7)	74 (5,6)	43 (3,3)	83 (6,3)
Pinus Pinaster **Ait.**	1 (0,1)	1 (0,1)	0 (0)	1 (0,1)	0 (0)	1 (0,1)	14 (1)	2 (0,1)	29 (2,1)	10 (0,7)	64 (4,8)	38 (2,8)	69 (5,2)	43 (3,2)	84 (6,5)	66 (5,1)
Pinus pinea **L.**	1 (0,1)	0 (0)	2 (0,1)	3 (0,2)	2 (0,1)	6 (0,5)	2 (0,2)	6 (0,4)	2 (0,1)	6 (0,4)	6 (0,4)	8 (0,6)	7 (0,5)	8 (0,6)	8 (0,6)	10 (0,7)
Pistacia lentiscus **L.**	89 (8,8)	141 (13,9)	22 (1,8)	33 (2,8)	37 (3,2)	52 (4,5)	71 (6,6)	87 (8)	119 (12,4)	167 (17,4)	114 (11,7)	159 (16,3)	125 (13)	163 (17)	128 (13,5)	173 (18,3)
Quercus ilex **L.**	3 (0,2)	3 (0,2)	5 (0,4)	12 (0,8)	13 (0,9)	22 (1,6)	19 (1,4)	28 (2)	20 (1,4)	36 (2,6)	34 (2,5)	52 (3,8)	38 (2,8)	49 (3,6)	39 (2,9)	51 (3,8)
Quercus pubescens **Willd.**	0 (0)	0 (0)	0 (0)	0 (0)	4 (0,3)	1 (0,1)	10 (0,8)	3 (0,2)	35 (2,5)	28 (2)	79 (6,1)	73 (5,7)	116 (9,3)	79 (6,3)	149 (12,6)	112 (9,5)
Quercus suber **L.**	0 (0)	4 (0,3)	1 (0,1)	6 (0,4)	4 (0,3)	11 (0,8)	7 (0,5)	13 (0,9)	21 (1,5)	21 (1,5)	57 (4,2)	38 (2,8)	72 (5,4)	37 (2,8)	102 (8)	60 (4,7)

III.3.3   Tree measurements

III.3.3.1 Stem diameter measurement

Basal diameter (10cm from root collar) was measured with a precision digital caliper in all individuals between January and February from 2014 to 2022. Autumn and early winter were avoided as several species typically grow in that period. Given the slow growth of some of the species and the frequent presence of multi stems or branches in the first 10 cm, the annual increments were often inaccurate. Actions to improve accuracy included the measurement of all stems when there were less than three, and the measurement of the main stem and assessment of number of stems within a diameter class when greater than three (some species, as *Phillyrea latifolia* L., may have more than 50 stems). The error due to the slow growth of individuals (below the accuracy of the measurement) did not allow us to evaluate the annual increment of all species with enough accuracy. Thus, for this article, we use: Basal Area (BA) in years 2019 and 2022 (BA19, BA22) and Basal Area Increments (BAI) from 2016 to 2019 and from 2016 to 2022 (BAI19-16 and BAI22-16).

Since in the first two years all blocks were irrigated and most of the replacement of dead individuals occurred in these first two years, the baseline for assessing increments is set to 2016.

III.3.4   Calculations of Net effect, Selection and complementarity

The Net Effect (NE) was decomposed in Selection and complementarity effects (SE &CE) following Loreau and Hector (2001). All Response variables were calculated using either total basal area as a proxy of aboveground biomass Basal area increments over the period 2016-2019 as a proxy of above ground productivity. The NE of a given mixture is calculated as:

$$NE = \sum Y - \bar{M}$$

where Y is the observed total biomass, or the biomass increment over a period, for each species and $\bar{M}$ is the average biomass, or average biomass increment, of all species when grown in monocultures.

The SE is calculated as:

$$SE = N \times Cov(M, \Delta RY)$$

where N is the number of species present in the mixture, M is a species' biomass in monoculture and ΔRY is the difference between the observed relative yield (RYo = Y/M) and the expected relative yield (RYe = 1/N). The CE is calculated as:

$$CE = \bar{M} \times N \times \overline{\Delta RY}$$

where $\overline{\Delta RY}$ is the average ΔRY of all the species in a mixture.

A positive NE occurs when a mixture produces more than expected from each species yield in the respective monoculture. A positive CE occurs when the observed relative yield is (Ryo) of a mixture is higher than that expected (RYe). A positive SE indicates that species with the largest yield in monocultures also have the largest RY in the mixture.

III.3.5   Statistical analyses

Linear mixed models (LMM), under the R (*R Core Team (2022)*, package = "*lme4*") (Pinheiro and Bates, 2000), were used to assess trait metrics influence on net, selection and complementary effects. This methodology was applied since it is particularly useful when using hierarchical data (nested random effects) which are not independent (e.g., diameter measures within the same plot, Bolker et al., 2009). Thus, in this study plot numbers and blocks were used as a categorical random effect to account for the potential influence of this grouping factors. A first model was used to test for the effects of SR, the irrigation treatment and their interaction on NE, CE and SE. In case of significant effect of SR, treatment and their interaction, NE, CE, SE were tested against zero in a one-sample t-test for each SR level and the effect of Treatment within each SR level. A second LMM was applied using, as fixed effects, the irrigation treatment (categorical) and community trait metrics such as Functional dispersion (FDis, Laliberte et al., 2010) and the community weighted mean (CWM, Garnier et al. 2004) calculated over several traits.

FDis, which is commonly used to assess FD influences on ecosystem functioning (Paquette & Messier, 2011; Verheyen et al., 2015), represents, in a trait space, the mean distance of individual species to the centroid of all species considering species abundances (here the species basal area within each plot). Instead, CWM is commonly used to represent dominant traits values within communities (Costa-Saura et al., 2019; Muscarella & Uriarte, 2016). Three FDis were calculated: one using all traits and two using the first and second axis of a PCA of all traits.

The first axis of PCA was mostly composed of leaf level traits (SLA, LLS, Pnmax and Nm) and WD. The second axis instead was mostly composed of traits related to water use strategy (PLC50, Huber value, WD, Mh and LA).

Table 5. *Trait measurements and their principal axis values.*

Traits	Axis 1	Axis 2	Axis 3
PLC50	0.05787	0.554313	0.044652
Huber	0.243854	0.393879	0.266426
WD	0.340178	0.369411	0.206025
Mh	0.289398	0.364786	0.250396
LA	0.292633	0.352242	0.047077
SDW	0.078329	0.257394	0.408521
Nm	0.370181	0.20483	0.278155
LLS	0.400069	0.119476	0.183728
Pnmax	0.364067	0.113235	0.232447
Gsmax	0.235901	0.087687	0.612826
SLA	0.400825	0.031551	0.334755

Subsequently, the effect of FDis and CWM were evaluated on NE, CE, SE in terms of either Basal Area increments over the period 2016-2019 or Basal Area in year 2019. FDis, Treatment, and CWM were used as fixed factors while Field and Plot were used as random variables. To evaluate the best combinations of variables, multiple models were tested using different combinations of FDis and of CWM.

The model structures thus were:

NE ~ A_val (*or +) Treat+ B_val+ SOIL+(1|Field)+ (1|Plot)

CE ~ A_val (*or +) Treat+ B_val+ SOIL+(1|Field)+ (1|Plot)

SE ~ A_val (*or +) Treat+ B_val+ SOIL+(1|Field)+ (1|Plot)

III.4 Results

SR had a significant effect on NE, CE, and SE irrespective of the dataset used (p values always < 0.001 and 0.05 respectively). The treatment was instead significant irrespective of the dataset (p values always < 0.05) only for NE. The SR: Treat interaction had no effect on NE, CE nor SE. (table 5).

Table 4. *Statistical Analysis of Net Diversity Effect (NE), Complementary Effect (CE), and Selection Effect (SE) across Different Datasets. This table presents the results of a statistical analysis for (NE), (CE), and (SE) in various datasets (BA19, BA22, BAI19-16, and BAI22-16). For each dataset type, the table includes degrees of freedom (d.f.), t-values, and p-values associated with the terms SR (Species Richness), Treat (Treatment), and their interaction SR:Treat. The statistical outcomes provide insights into the significance of these effects on the respective datasets.*

Dataset type	Term	NE			CE			SE		
		d.f.	t value	p	d.f.	t value	p	d.f.	t value	p
BA19	SR	184	419.441	**4.25E-05**	1.867.778	2.732.467	**0.00689**	1.814.628	3.384.328	**0.000874**
BA19	Treat	7.339.949	1.762.374	**0.062169**	1.866.516	1.177.804	0.240374	1.889.426	1.211.116	0.240782
BA19	SR:Treat	184	-0.70019	0.48469	1.879.423	0.191944	0.847993	1.834.523	-111.258	0.267343
BA22	SR	187.385	5.128.421	**7.25E-07**	1.863.234	3.765.544	**0.000223**	1.825.646	3.798.461	**0.000198**
BA22	Treat	1.872.659	2.490.101	**0.013642**	1.862.055	2.450.672	**0.015183**	2.896.409	1.199.001	0.240245
BA22	SR:Treat	187.985	-138.707	0.167062	1.877.859	-0.85744	0.392295	1.839.387	-116.406	0.245909
BAI19-16	SR	188	417.819	**4.5E-05**	1.798.444	2.497.626	**0.013401**	1.812.627	3.262.983	**0.001318**
BAI19-16	Treat	188	1.868.542	**0.049242**	5.248.096	1.124.641	0.265859	1.596.184	1.131.567	0.274531
BAI19-16	SR:Treat	188	-0.79573	0.427193	1.822.739	-0.07994	0.936376	1.833.008	-0.92118	0.358165
BAI22-16	SR	187.185	5.138.018	**6.94E-07**	1.804.018	2.851.367	**0.004861**	184	2.930.338	**0.003815**
BAI22-16	Treat	187.06	2.572.632	**0.010868**	5.086.848	1.311.441	0.195597	2.357.558	1.259.719	0.220099
BAI22-16	SR:Treat	1.879.999	-14.839	0.139511	1.826.992	-0.40591	0.685287	184	-116.675	0.24482

Since the results were extremely similar for all the 4 datasets used, further analysis only focuses on the BAI22-16 dataset (Basal Area increments between 2022 and 2016). NE was higher in the irrigated blocks as compared to the control blocks for SR levels 2 and 4 (p<0.01) but not for SR=6.

The predictors of the models with the lowest Akaike Information Criterion value (AIC) resulted to be the same for all three dependent variables, NE, CE, SE. Specifically these were FDis.1 and CWM.WD. NE was significantly affected by FDis.1 (p<0.0001), Treatment (p=0.018), and CWM.WD (p<0.0001). Similarly, CE was significantly affected by FDis.1 (p=0.034), Treatment (p=0.044), and CWM.WD (p<0.002). SE instead was not affected by the Irrigation treatment but only by FDis.1 (p=0.008) and by CWM.WD (p<0.0001). The interaction between FDis.1 and Irrigation Treatment was never significant. The model without the interaction had a higher AIC and thus the model could not be simplified by removing the interaction term.

Table 5. *Statistical Analysis of Net Diversity Effect (NE), Complementary Effect (CE), and Selection Effect (SE) for Specific Ecological Parameters in the BAI22-16 Dataset. This table presents the statistical analysis results for (NE), (CE), and (SE) in the BAI22-16 dataset. The table includes degrees of freedom (df), t-values, and p-values associated with specific ecological parameters, including FDis.1 (Functional Dispersion), Treat (Treatment), CWM.WD (Community-Weighted Mean of Wood Density), and the interaction term FDis.1:Treat. The statistical outcomes provide insights into the significance of these effects on the BAI22-16 dataset for the specified ecological parameters.*

		NE			CE			SE		
Dataset	term	df	t value	p	df	t value	p	df	t value	p
BAI22-16	FDis.1	187	4.736.236	**4.29E-06**	1.819.081	2.132.117	**0.034339**	1.836.777	2.670.541	**0.008252**
BAI22-16	Treat	187	2.377.773	**0.018426**	1.123.137	2.039.845	**0.043712**	5.957.701	0.235638	0.814522
BAI22-16	CWM.WD	187	-901.869	**2.27E-16**	177.522	-31.096	**0.002183**	1.836.724	-618.418	**3.95E-09**
BAI22-16	FDis.1:Treat	187	-15.764	0.116624	1.812.471	-151.155	0.132391	1.834.101	-0.00813	0.993519
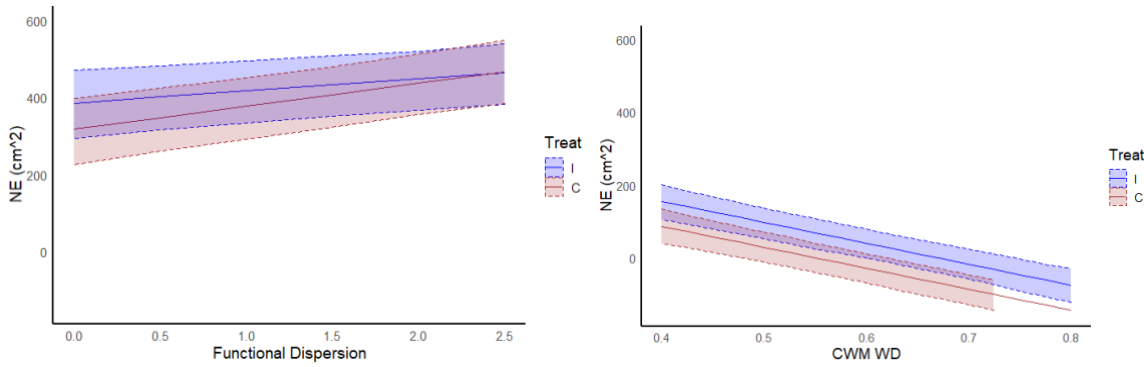
Figure 3. *The relationship between the Net Effect (NE) on Basal Area increments over the years 2022 and 2016 and Functional Dispersion (A) and Community Weighted Mean Wood Density (CWM WD). Solid lines represent the mean response and dashed lines the confidence intervals.*
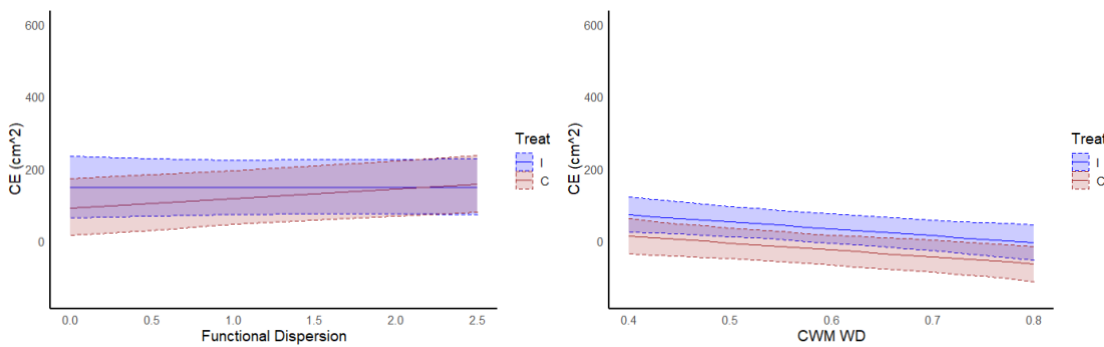
Figure 4. *The relationship between the Complementarity Effect (CE) on Basal Area increments over the years 2022 and 2016 and Functional Dispersion (A) and Community Weighted Mean Wood Density (CWM WD). Solid lines represent the mean response and dashed lines the confidence intervals.*
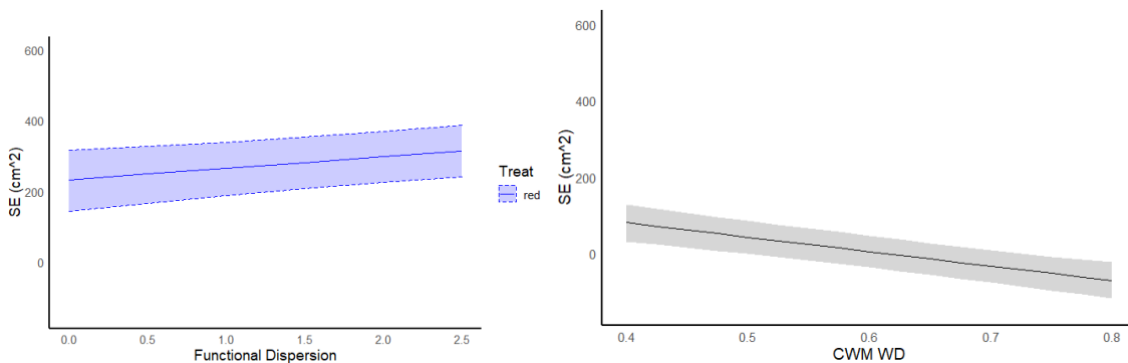
Figure 5. *The relationship between the Selection Effect (SE) on Basal Area increments over the years 2022 and 2016 and Functional Dispersion (A) and Community Weighted Mean Wood Density (CWM WD). Solid lines represent the mean response and dashed lines the confidence intervals.*

In the control plots, NE ranged from -26.8 for the QUSU/ARUN (although not significant) to maximum of 180.16 for the QUIL/OLEU/ARUN/PIPA/PIPE/ACMO mixture. Only five values of NE were below zero all of them not significant. 13 (out of 32) NE values were significantly greater than zero (Figure 6). CE ranged from -40.3 for the QUSU/PIHA mixture (not significant) to a maximum of 102.57 for the QUIL/OLEU/ARUN/PIPA/PIPE/ACMO mixture. In total, 9 out of 32 CE values were negative but only one significant. The remaining CE values were greater than 0 but only 3 significantly (Figure 6). SE ranged from -19.5 for the PIPA/PIHA to 126.5 for the QUSU/PHLA/PIPEACMO. 4 SE values were negative but none of them significantly, of the remaining 28 SE values 15 were significantly greater than zero (Figure 6).
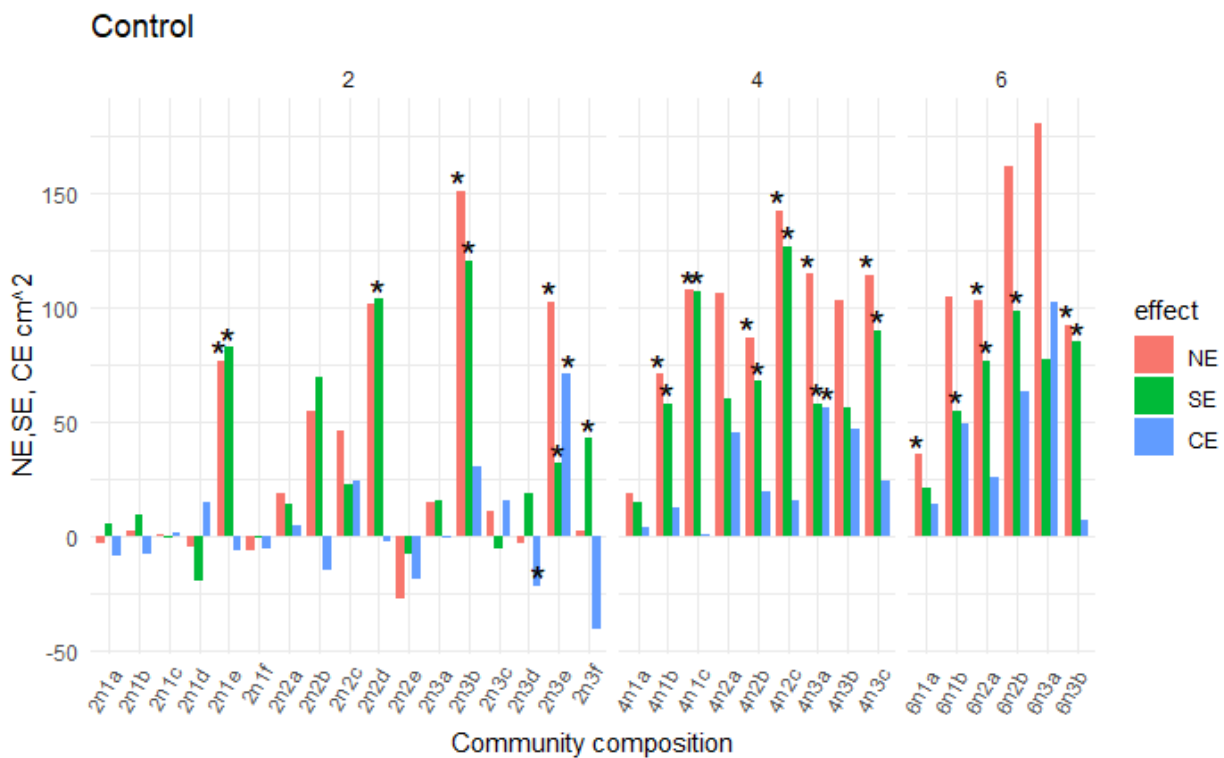
Figure 6. *Comparison of Net Diversity Effect (NE), Selection Effect (SE), and Complementary Effect (CE) in Tree Community Composition under Non-Irrigation (Control) Conditions.*

In the irrigated plots, NE never resulted to be significantly lower than 0 and was significantly above 0 in a total of 11 different community compositions. NE ranged from 0 for the OLEU/PHLA to maximum of 190.5 for the OLEU/PILE/PIPA/QUPU mixture (Figure 7). CE ranged from -27.5 for the ARUN/PILE/PIPA/PIHA mixture (not significant) to a maximum of 115.6 for the PIHA/ACMO mixture. In total, 9 out of 32 CE values were negative but not significant. The remaining CE values were greater than 0 but only 3.

Significantly (Figure 7). SE ranged from -58.16 for the PIPA/PIHA to 192.8 for the OLEU/PILE/PIPA/QUPU. 6 SE values were negative but none of them significantly, of the remaining 26 SE values 6 were significantly greater than zero (Figure 7).
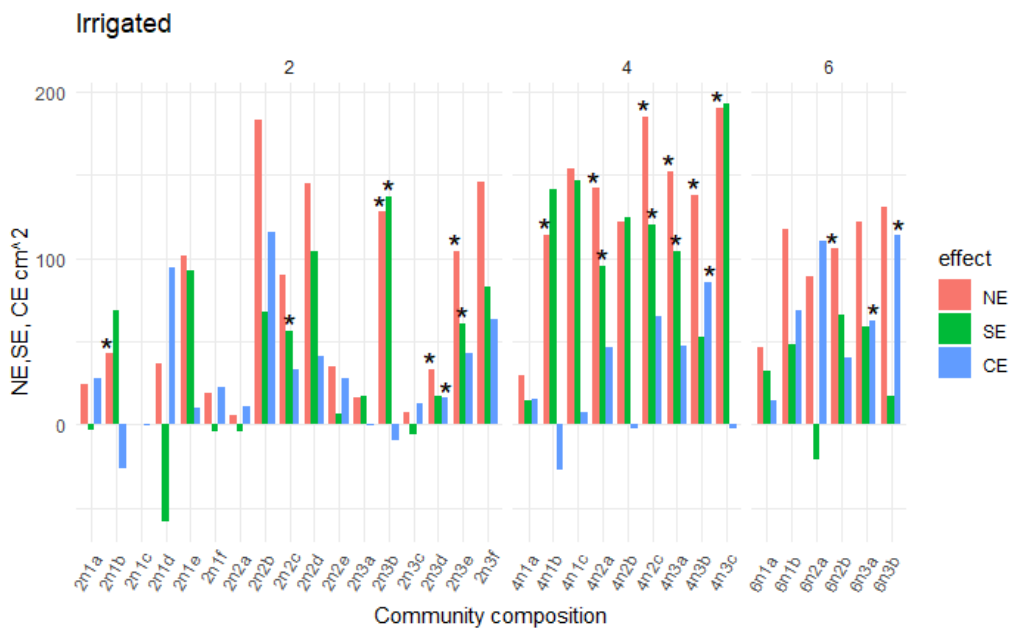
Figure 7. *Comparison of Net Diversity Effect (NE), Selection Effect (SE), and Complementary Effect (CE) in Tree Community Composition under Irrigation Conditions.*

III.5 Discussion

The number of tree diversity manipulation experiments and other studies investigating the biodiversity and ecosystem function relationships in Mediterranean ecosystems, or even seasonally dry ecosystems is very limited compared to those focusing on temperate and boreal biomes. It is thus relevant to report that the results of this work found a positive relationship between both SR and FDis with aboveground biomass accumulation similarly to what found in other biomes and in agreement with those few works in seasonally dry ecosystems.

SR is a commonly used metric in BEF studies also because it is easy to assess, however FD and CWM are increasingly being used as trait datasets increase, but also because they offer more insights on the ecological mechanism that explain the BEF relationships and in general tend to explain the observed variance in biodiversity effects better than SR. Such is the case also in this work where the model with FDis and CWM WD outperforms that with SR, supporting the idea that what really matters for ecosystem functions is not the number of species but how much they differ in terms of resource use strategies.

It is often debated that complementarity in water resource use could explain some observed positive effects when mixing species especially in dry ecosystems or in extreme drought events. For example, a temporal complementarity could be observed when mixing species on the two extremes of the isohydric-anysohydric gradient. These species tend to have contrasting GSmax as noted by Sperry et al. (2017), hydraulic conductivity, PLC50, Huber values and the outcome of the behavior is that they either tend to have a low but constant transpiration rate throughout the year or have high transpiration values at high water availability but then close their stomata when the soil is moderately dry.

Mixing species could lead to a more efficient partitioning of water resources, enhancing water use efficiency at the community level. This notion aligns with findings from Grossiord, who has demonstrated the critical role of species diversity in modulating community-level responses to water availability and drought (Grossiord, 2018). However, in our experiment, models focusing solely on 'water-related' traits or those incorporating a broader range of traits did not significantly impact the analysis, as indicated by higher AIC values. This suggests that, in the context of this specific experiment, differences in water use strategies might not have been as pivotal. This observation stands in contrast to/agreement with Grossiord's work, which underscores the importance of diverse water

use strategies for ecosystem resilience, indicating that the relevance of such strategies may vary depending on experimental design and ecological context.

As found in all species manipulation experiments, the general positive effect of FDis on NE, CE, and SE is not sufficient to explain the observed variability with both high and low effects observed along the FDis gradient and further suggesting that more work is needed to understand and predict exactly when the mixing of species yield a positive effect on the desired function. As in other works, even accounting for non-significant effects, communities with negative NE were very few and, when negative, the effect was mild. This should encourage the mixing of species as the worst outcome is likely not to cause any yield loss while probably still improving other ecosystem functions including resilience to climate change.

Our analysis challenges and expands upon the conventional Stress Gradient Hypothesis (SGH). Our investigation reveals that the interplay between biodiversity, functional diversity, and irrigation does not conform neatly to the predictions of the SGH. This deviation, partially aligning with findings by Smith et al. (2020) and contrasting with Johnson and Lee (2021), unveils a complex and nuanced ecological interaction. The SGH does not explicitly say if higher intercept or higher slopes of NE or CE effects should be expected in dryer conditions. Simlarly to Belluau et al. 2020 that have ocndicted a simalr experiment and analsys in boreal systems, our results do not confirm the SGH and to some extend suggest the contrary as both NE, CE, and SE are higher in the irrigated blocks. However, we do observe also some differences with Belluau et al., 2020 as they also found a higher slope for FDis in the irrigated blocks while the intercept of the two treatments was similar on eventually higher in the dry treatment, whereas in this Mediterranean experiment we found that the slope of the response did not differ between treatments.

While these results add to the extensive literature promoting mixed plantations and mixed stands in general they also entail, in a climate change perspective, that increasing droughts will reduce the NE of diversity that we have benefited from so far at least in terms of above-ground yields.

These findings prompt a reevaluation of current ecological models and theories, particularly in the context of the swiftly changing climatic conditions impacting Mediterranean ecosystems. The implications of this study extend well beyond theoretical insights, influencing practical strategies in biodiversity conservation and ecosystem management under environmental stressors. However, it is crucial to acknowledge the limitations of our study, including the selection of species, the scale of

the study, and the controlled experimental conditions, which advise caution in overgeneralizing these results. This highlights the necessity for more comprehensive research in varied ecological settings to corroborate these findings.

Future research activities should aim to replicate and extend these observations across diverse Mediterranean ecosystems, encompassing a wider array of species, varying stress factors, and longer time frames. Such investigations are essential to fully grasp the resilience and adaptability of these ecosystems to climatic changes. Our study, Reflecting the comprehensive insights of Gomez and Patel (2019) and building upon the foundational work of Alvarez and Chen (2018), underscores the need for dynamic and adaptive management approaches. By challenging established paradigms and introducing novel perspectives on species interactions and ecosystem functionality, this research marks a significant advancement in our understanding of Mediterranean tree communities amidst global environmental shifts.

III.6 Conclusion

Our study highlights the importance of biodiversity in shaping ecosystem dynamics, particularly in the context of water availability. Trait-based metrics, like Functional Dispersion and Community Weighted Mean Wood Density, provide valuable insights into the functional diversity and structure of tree communities.

Our findings challenge the traditional Stress Gradient Hypothesis, suggesting a more complex relationship between biodiversity, functional diversity, and irrigation in Mediterranean ecosystems. This has practical implications for conservation and management.

Overall, our work contributes to a deeper understanding of Mediterranean tree communities and underscores the need for adaptive management strategies in the face of global environmental changes.

References

Ali, E., W. Cramer, J. Carnicer, E. Georgopoulou, N.J.M. Hilmi, G. Le Cozannet, and P. Lionello, 2022: Cross-Chapter Paper 4: Mediterranean Region. In: Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C.Roberts, M.Tignor, E.S.Poloczanska, K.Mintenbeck, A.Alegría, M.Craig, S.Langsdorf, S.Löschke, V.Möller, A. Okem, B. Rama (eds.)]. Cambridge University Press, Cambridge, UK and New York, NY, USA, pp. 2233–2272, doi:10.1017/9781009325844.021.

Ammer, C. (2019). Diversity and forest productivity in a changing climate. New Phytologist, 221, 50–66. https://doi.org/10.1111/nph.15263

Armas, C. & Pugnaire, F.I. (2005). Plant interactions govern population dynamics in a semiarid plant community. J. Ecol., 93, 978–989.

Balvanera, P., Pfisterer, A.B., Buchmann, N., He, J.-S., Nakashizuka, T., Raffaelli, D. and Schmid, B. (2006), Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecology Letters, 9: 1146-1156. https://doi.org/10.1111/j.1461-0248.2006.00963.x

Belluau et al. (2021) Overyielding in young tree communities does not support the stress-gradient hypothesis and is favoured by functional diversity and higher water availability, Journal of Ecology, DOI: 10.1111/1365-2745.13602

Bertness, M. D., and Callaway, R. (1994). Positive interactions in communities. Trends in Ecology and Evolution, 9(5), 191-193.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., and White, J. S. S. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology and Evolution, 24(3), 127–135. https://doi.org/10.1016/j.tree.2008.10.008

Brooker, R.B. (2006). Plant–plant interactions and environmental change. New Phytol., 171, 271–284.

Brooker, R.W., Maestre, F.T., Callaway, R.M., Lortie, C.L., Cavieres, L.A., Kunstler, G. et al. (2008). Facilitation in plant communities: the past, the present, and the future. J. Ecol., 96, 18–34

Cadotte, M.W., Carscadden, K. and Mirotchnick, N. (2011), Beyond species: functional diversity and the maintenance of ecological processes and services. Journal of Applied Ecology, 48: 1079-Cardinale, B. J., Srivastava, D. S., Duffy, J. E., Wright, J. P., Downing, A. L., Sankaran, M., & Jouseau, C. (2006). Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature, 443(7114), 989-9921087. https://doi.org/10.1111/j.1365-2664.2011.02048.x

Cardinale, B. J., Matulich, K. L., Hooper, D. U., Byrnes, J. E., Duffy, E., Gamfeldt, L., Balvanera, P., O'Connor, M. I., & Gonzalez, A. (2011). The functional role of producer diversity in ecosystems. American journal of botany, 98(3), 572–592. https://doi.org/10.3732/ajb.1000364

Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., Narwani, A., Mace, G.M., Tilman, D., A.Wardle, D., Kinzig, A.P., Daily, G.C., Loreau, M., Grace, J.B., Larigauderie, A., Srivastava, D.S., and Naeem, S. (2012). Corrigendum: biodiversity loss and its impact on humanity. Nature 489, 59–67.

Castanho, C.T., Oliveira, A.A. & Prado, P.I. (2012). The importance of plant life form on spatial associations along a subtropical coastal dune gradient. J. Veg. Sci., 23, 952–961

Convention on Biological Diversity (CBD). (2020). key international instrument for sustainable development, https://www.cbd.int/doc/c/abb5/591f/2e46096d3f0330b08ce87a45/wg2020-03-03-en.pdf.

Costa-Saura, J. M., Trabucco, A., Spano, D., & Mereu, S. (2017). Environmental filtering drives community specific leaf area in Spanish forests and predicts relevant changes under future climatic conditions. Forest Ecology and Management, 405, 1–8. https://doi.org/https://doi.org/10.1016/j.foreco.2017.09.023

Costa-Saura, J. M., Trabucco, A., Spano, D., and Mereu, S. (2019). A height-wood-seed axis which is preserved across climatic regions explains tree dominance in European forest communities. Plant Ecology, 220(4–5). https://doi.org/10.1007/s11258-019-00928-x

Cowles, J. M., P. D. Wragg, A. J. Wright, J. S. Powers, and D. Tilman. 2016. Shifting grassland plant community structure drives positive interactive effects of warming and diversity on aboveground net primary productivity. Global Change Biology 22:741–749.

Díaz, S. & Cabido, M. (2001). Vive la difference: plant functional diversity matters to ecosystem processes. Trends Ecol. Evol., 16, 646–655.

Elton, C. S. (1927). Animal Ecology. Earth Sciences: GENERAL EARTH SCIENCES, Oxford University Press.

Forrester, D. I., & Bauhus, J. (2016). A review of processes behind diversity—productivity relationships in forests. Current Forestry Reports, 2, 45–61. https://doi.org/10.1007/s40725-016- 0031-2

Gazol, A., Camarero, J. J., Sangüesa-Barreda, G., and Vicente-Serrano, S. M. (2018). Post-drought Resilience After Forest Die-Off: Shifts in Regeneration, Composition, Growth and Productivity. Frontiers in plant science, 9, 1546. https://doi.org/10.3389/fpls.2018.01546

Goldberg, D., Novoplansky, A., Gurevitch, J. & Stewart-Oaten, A. (1999). Empirical approaches to quantifying interaction intensity: competition and facilitation along productivity gradients. Ecology, 80, 1118–1131.

Gomez-Aparicio, L. (2009). The role of plant interactions in the restoration of degraded ecosystems: a meta-analysis across life-forms and ecosystems. J. Ecol., 97, 1202–1214.

Gravel, D., Bell, T., Barbera, C., Bouvier, T., Pommier, T., Venail, P., et al. (2011). Experimental niche evolution alters the strength of the diversity-productivity relationship. Nature, 469, 89–92.

Gross, N., Liancourt, P., Choler, P., Suding, K.N. & Lavorel, S. (2010). Strain and vegetation effects on local limiting resources explain the outcomes of biotic interactions. Perspect. Plant Ecol. Evol., 12, 9–19.

Grossiord, C. (2018). Having the right neighbors: how tree species diversity modulates drought impacts on forests. New Phytologist, 228. https://doi.org/10.1111/nph.15667

Hajek et al. 2022. Mutually inclusive mechanisms of drought-induced tree mortality. Global Change Biology, 28, 10, 3365-3378.

Harmon, L. J., Matthews, B., Des Roches, S., Chase, J. M., Shurin, J. B., & Schluter, D. (2009). Evolutionary diversification in stickleback affects ecosystem functioning. Nature, 458(7242), 1167–1170. https://doi.org/10.1038/nature07974

He, Q., Bertness, M. D., & Altieri, A. H. (2013). Global shifts towards positive species interactions with increasing environmental stress. Ecology Letters, 16(5), 695–706. https://doi.org/10.1111/ele.12080

He, Q., Cui, B., Bertness, M.D. & An, Y. (2012). Testing the importance of plant strategies on facilitation using congeners in a coastal community. Ecology, 93, 2023–2029.

Hisano, M., Searle, E. B., & Chen, H. Y. H. (2018). Biodiversity as a solution to mitigate climate change impacts on the functioning of forest ecosystems. Biological Reviews, 93, 439–456. https://doi.org/10.1111/ brv.12351

Holzapfel, C., Tielb€orger, K., Pavag, H.A., Nigel, J. & Sternbeug, H. (2006). Annual plant–shrub interactions along an aridity gradient. Basic Appl. Ecol., 7, 268–279.

Hooper, D. U., Adair, E. C., Cardinale, B. J., Byrnes, J. E. K., Hungate, B. A., Matulich, K. L., Gonzalez, A., Duffy, J. E., Gamfeldt, L., & O'Connor, M. I. (2012). A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature, 486(7401), 105–108. https://doi.org/10.1038/nature11118

Jiang L, Wan s, Li L (2009) species diversity and productivity: why do results of diversity-manipulation experiments differ from natural patterns? J ecol 97:603–608

Kelty, M. (2006). The role of species mixtures in plantation forestry. Forest Ecology and Management, 233, 195–204. https://doi.org/10.1016/j. foreco.2006.05.011

Laliberte, E., Legendre, P., Ecology, S., and January, N. (2010). A distance-based framework for measuring functional diversity from multiple traits A distance-based framework for measuring

from multiple traits functional diversity. Ecology, 91(1), 299–305. https://doi.org/10.1890/08-2244.1

Leopold, A. (1949). A Sand County Almanac. Oxford University Press.

Lionello, P., Abrantes, F., Gacic, M., Planton, S., Trigo, R., and Ulbrich, U. (2014). The climate of the Mediterranean region: research progress and climate change impacts. Regional Environmental Change, 14(5), 1679–1684. https://doi.org/10.1007/s10113-014-0666-0

Lortie, C.J. & Callaway, R.M. (2006). Re-analysis of meta-analysis: support for the stress-gradient hypothesis. J. Ecol., 94, 7–16.

Loreau, M., & Hector, A. (2001). Partitioning selection and complementarity in biodiversity experiments. Nature, 412(July), 72–76. https://doi.org/10.1038/35083573

Maestre, F.T., Callaway, R.M., Valladares, F. & Lortie, C.J. (2009). Refining the stress-gradient hypothesis for competition and facilitation in plant communities. J. Ecol., 97, 199–205.

Maestre, F.T., Valladares, F. & Reynolds, J.F. (2005). Is the change of plant– plant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments. J. Ecol., 93, 748–757

Maestre, F.T. & Cortina, J. (2004). Do positive interactions increase with abiotic stress? A test from a semi-arid steppe. Proc. Biol. Sci., 271, S331–S333.

Maxwell et al., 2022. Tree species identity drives nutrient use efficiency in young mixed-species plantations, at both high and low water availability. Functional Ecology. DOI: 10.1111/1365-2435.14109

Mendoza, I., Zamora, R., & Castro, J. (2009). A seeding experiment for testing tree-community recruitment under variable environments: Implications for forest regeneration and conservation in Mediterranean habitats. Biological Conservation, 142(7), 1491–1499. https://doi.org/https://doi.org/10.1016/j.biocon.2009.02.018

Michalet, R. (2006). Is facilitation in arid environments the result of direct or complex interactions? New Phytol., 169, 1–2.

Mokany, K., Ash, J. & Roxburgh, S. (2008). Functional identity is more important than diversity in influencing ecosystem processes in a temperate native grassland. J. Ecol., 96, 884–893.

Muscarella, R., and Uriarte, M. (2016). Do community-weighted mean functional traits reflect optimal strategies? Proceedings of the Royal Society B, 283(March), 20152434. https://doi.org/10.1098/rspb.2015.2434

Naeem S. 1998. Species redundancy and ecosystem reliability. Conserv Biol. 12:39–45. doi:10.1111/j.1523-1739.1998.96379.x.

Naeem, S. (2002). Disentangling the impacts of diversity on ecosystem functioning in combinatorial experiments. Ecology, 83, 2925–2935.

Paquette, A., and Messier, C. (2011). The effect of biodiversity on tree productivity: from temperate to boreal forests. Global Ecology and Biogeography, 20(1), 170–180. https://doi.org/10.1111/j.1466-8238.2010.00592.x

Pinheiro, J.C., and Bates, D.M. (2000). Mixed-effects Models in S and S-PLUS. Springer

Poorter, L., van der Sande, M.T., Thompson, J., Arets, E.J.M.M., Alarcón, A., Álvarez-Sánchez, J., Ascarrunz, N., Balvanera, P., Barajas-Guzmán, G., Boit, A., Bongers, F., Carvalho, F.A., Casanoves, F., Cornejo-Tenorio, G., Costa, F.R.C., de Castilho, C.V., Duivenvoorden, J.F., Dutrieux, L.P., Enquist, B.J., Fernández-Méndez, F., Finegan, B., Gormley, L.H.L., Healey, J.R., Hoosbeek, M.R., Ibarra-Manríquez, G., Junqueira, A.B., Levis, C., Licona, J.C., Lisboa, L.S., Magnusson, W.E., Martínez-Ramos, M., Martínez-Yrizar, A., Martorano, L.G., Maskell, L.C., Mazzei, L., Meave, J.A., Mora, F., Muñoz, R., Nytch, C., Pansonato, M.P., Parr, T.W., Paz, H., Pérez-García, E.A., Rentería, L.Y., Rodríguez-Velazquez, J., Rozendaal, D.M.A., Ruschel, A.R., Sakschewski, B., Salgado-Negret, B., Schietti, J., Simões, M., Sinclair, F.L., Souza, P.F., Souza, F.C., Stropp, J., ter Steege, H., Swenson, N.G., Thonicke, K., Toledo, M., Uriarte, M., van der Hout, P., Walker, P., Zamora, N. and Peña-Claros, M. (2015), Carbon storage in tropical forests. Global Ecology and Biogeography, 24: 1314-1328. https://doi.org/10.1111/geb.12364

Ratcliffe, S., Wirth, C., Jucker, T., van der Plas, F., Scherer-Lorenzen, M., Verheyen, K., Allan, E., Benavides, R., Bruelheide, H., Ohse, B., Paquette, A., Ampoorter, E., Bastias, C. C., Bauhus, J., Bonal, D., Bouriaud, O., Bussotti, F., Carnol, M., Castagneyrol, B., … Baeten, L. (2017).
95

Biodiversity and ecosystem functioning relations in European forests depend on environmental context. Ecology Letters, 20(11), 1414–1426. https://doi.org/10.1111/ele.12849

Reich, P.B. (2012). Key canopy traits drive forest productivity. Proc. Biol. Sci., 279, 2128–2134

Reiss J, Bridle JR, Montoya JM, Woodward G (2009) emerging horizons in biodiversity and ecosystem functioning research. Trends ecol evol 24:505–514

Rogelj, J., den Elzen, M., and Höhne, N. (2016). Paris Agreement climate proposals need a boost to keep warming well below 2 °C. Nature 534, 631–639. https://doi.org/10.1038/nature18307

Roscher, C., Schumacher, J., Gubsch, M., Lipowsky, A., Weigelt, A., Buchmann, N., et al. (2012). Using plant functional traits to explain diversity-productivity relationships. PLoS ONE, 7, e36760.

Sperry, J. S., Venturas, M. D., Anderegg, W. R. L., Mencuccini, M., Mackay, D. S., Wang, Y., & Love, D. M. (2017). Predicting stomatal responses to the environment from the optimization of photosynthetic gain and hydraulic cost. Plant, Cell & Environment, 40(6), 816-830.

Steinauer, K., D. Tilman, P. D. Wragg, S. Cesarz, J. M. Cowles, K. Pritsch, P. B. Reich, W. W. Weisser, and N. Eisenhauer. (2015). Plant diversity effects on soil microbial functions and enzymes are stronger than warming in a grassland experiment. Ecology 96:99–112.

Sthultz, C.M., Gehring, C.A. & Whitham, T.G. (2007). Shifts from competition to facilitation between a foundation tree and a pioneer shrub across spatial and temporal scales in a semiarid woodland. New Phytol., 173, 135–145.

Suding, K., Higgs, E., Palmer, M., Callicott, J. B., Anderson, C. B., Baker, M., Gutrich, J. J., Hondula, K. L., LaFevor, M. C., Larson, B. M. H., Randall, A., Ruhl, J. B., and Schwartz, K. Z. S. (2015). Committing to ecological restoration. Science, 348(6235), 638–640. https://doi.org/10.1126/science.aaa4216

Tielborger, K. & Kadmon, R. (2000). Temporal environmental variation tips the balance between facilitation and interference in desert plants. Ecology, 81, 1544–1553.

Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., & Siemann, E. (1997). The influence of functional diversity and composition on ecosystem processes. Science, 277(5330), 1300-1302.

Tobner et al. (2014) Advancing biodiversity–ecosystem functioning science using high-density tree-based experiments over functional diversity gradients. Oecologia. DOI 10.1007/s00442-013-2815-4

Tylianakis, J. M., Didham, R. K., Bascompte, J., and Wardle, D. A. (2008). Global change and species interactions in terrestrial ecosystems. Ecology Letters, 11(12), 1351–1363. https://doi.org/https://doi.org/10.1111/j.1461-0248.2008.01250.x

Verheyen, K., Vanhellemont, M., Auge, H., Baeten, L., Baraloto, C., Barsoum, N., Bilodeau-Gauthier, S., Bruelheide, H., Castagneyrol, B., Godbold, D., Haase, J., Hector, A., Jactel, H., Koricheva, J., Loreau, M., Mereu, S., Messier, C., Muys, B., Nolet, P., and Scherer-Lorenzen, M. (2015). Contributions of a global network of tree diversity experiments to sustainable forest plantations. Ambio. https://doi.org/10.1007/s13280-015-0685-1

Vilà M, Vayreda J, Gracia C, Ibáñez JJ (2003) Does tree diversity increase wood production in pine forests? Oecologia 135:299–303

Violle C et al (2007) Let the concept of trait be functional! Oikos 116:882

Wang, J., Zhang, C. B., Chen, T., & Li, W. H. (2013). From selection to complementarity: The shift along the abiotic stress gradient in a controlled biodiversity experiment. Oecologia, 171, 227–235. https://doi.org/10.1007/s0044 2- 012-2400-2

Walker BH. 1992. Biodiversity and ecological redundancy. Conserv Biol. 6:18–23. doi:10.1046/j.1523-1739.1992.610018.x.

Wright, A., S. A. Schnitzer, and P. B. Reich. 2014. Living close to your neighbors—the importance of both competition and facilitation in plant communities. Ecology 95:2213–2223.

Xu S, Eisenhauer N, Ferlian O, Zhang J, Zhou G, Lu X, Liu C, Zhang D. 2020. Species richness promotes ecosystem carbon storage: evidence from biodiversity-ecosystem functioning experiments. Proc Biol Sci. 287:20202063. doi:10.1098/rspb.2020. 2063

IV. Chapter 4 Ecological Benefits of Mixed Forest Plantations in the Mediterranean ecosystem: A Summary of Recent Studies

IV.1 Abstract

Forests are essential for the health and well-being of the planet, providing numerous ecological, social, and economic benefits. However, traditional monoculture forestry practices have often resulted in ecological degradation and loss of biodiversity. In response, many researchers and practitioners have turned to mixed forest plantations as a more sustainable alternative. All previous work on the relationship between Biodiversity and Ecosystem functions point to a clear general positive relationship between species richness or Functional diversity and growth. Nevertheless, the relationship is not always true as it is often context specific and depends on the community composition. Indeed, the outcome of the interactions among neighboring species is the result of numerous interaction pathways that range from sharing mycorrhizal networks to resource portioning e.g. differentiated root systems that use water at different depths. Understanding these mechanisms is where research is focusing as their understanding would allow to accurately predict and plan the outcome of a new plantation (or NBS) in terms of carbon sequestration and possibly other ecosystem functions. Here we give a brief overview of research activities focused on at least one mechanism that either took place only in Macomer or are a part of a multi-site research. In the latter case, the activities were undertaken under the umbrella of the MixForChange and CAMBIO projects which are briefly summarized.

 The TreeDivNet community is very active and it is common for different researchers to use data form the IDENT-MACOMER site and visit to collect data them self. In the last 3 years the collaboration has intensified within the MixForChange and Cambio projects. This chapter describes some of the activities that have taken place in IDENT-MACOMER to which the candidate has taken part. Some of these activities were specific for the site while others were cross site activities. By synthesizing the key findings of these studies, we aim to provide a comprehensive overview of the potential benefits of mixed forest plantations and their implications for ecological restoration and climate change mitigation.

Mediterranean ecosystem; carbon sequestration; climate change mitigation; mixed forest plantations; ecosystem services

Introduction

IV.2 Overview of Two Pioneering Climate Change Mitigation and Adaptation Projects

Increasing interest in interpreting the complex relationships between biodiversity and ecosystem functionality highlights the critical need to uncover the ecological processes that drive these connections. Recent research, such as the findings presented in Depauw et al. (2024), highlights the significant role of species diversity, particularly through tree species mixing, in enhancing tree performance and overall ecosystem health. This body of work not only contributes to our fundamental comprehension of biodiversity's impact on ecosystem function but also paves the way for translating theoretical insights into actionable forest management strategies, thereby fostering more resilient and productive ecosystems.

In a world facing ever-increasing challenges associated with global change, two innovative projects have emerged as signs of hope, promising tangible solutions rooted in the very heart of nature itself. The "Mix for Change project", abbreviated as MFC, stands as a powerful advocate for the role of mixed-species forest plantations as nature-based tools to combat the causes and consequences of climate change. Simultaneously, the "CAMBIO" project, which stands for Climate change Adaptation and Mitigation with Biodiverse forest plantations, is dedicated to synthesizing knowledge from tree diversity experiments worldwide, all with the aim of optimizing the functionality of planted forests within the context of our rapidly changing climate. Together, these two initiatives offer science-based recommendations and guidelines that resonate with forest owners, managers, and policy-makers, uniting their efforts to create a sustainable, resilient, and ecologically responsible future. In this chapter, we dig into the experiments conducted under these two groundbreaking projects, exploring their potential to initiate a new era of forest management in the face of climate uncertainty.

CAMBIO and MFC are composed to build on the powerful foundation provided by TreeDivNet, which stands as the largest network of biodiversity experiments worldwide. Comprising 29 experiments including IDENT-MACOMER, this network has witnessed the planting of an astounding 1,239,576 trees across a total surface area of 859 hectares. Each of these experiments was
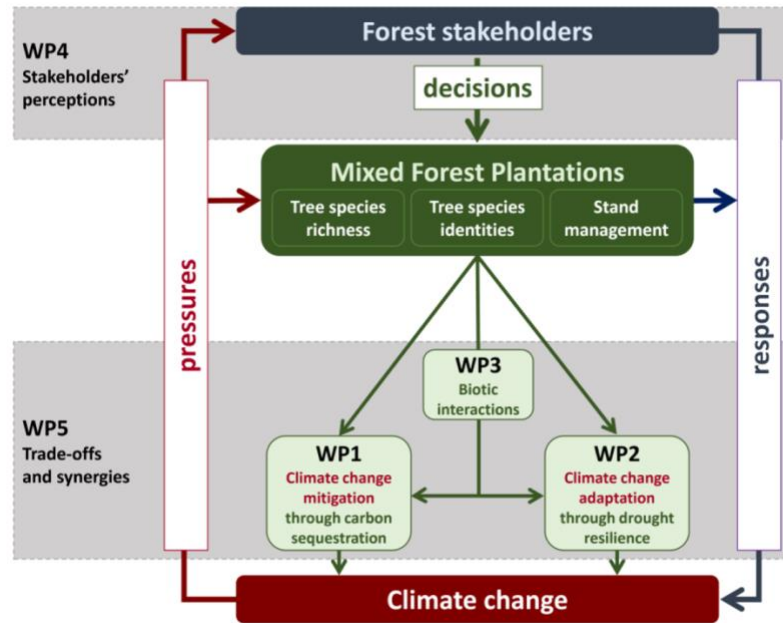
meticulously designed to probe the impacts of tree diversity and composition on the early stages of forest development. As such, TreeDivNet proudly holds the title of the largest research facility globally, dedicated to the comprehensive examination of forest ecosystem functioning within plantations. It is from this formidable vantage point that CAMBIO and MFC take their lead, embarking on a mission to further our understanding of these vital ecosystems and fortify our efforts in the fight against climate change.

IV.2.1 Mix for Change project

The MFC project (https://mixforchange.eu/language/en/) relies on a unique global network of forest biodiversity experiments (TreeDivNet), which are based on a common, statistically sound design that allows for the effects of tree species composition on ecosystem services and functions to be tested by providing a mechanistic understanding of why and how tree diversity, species identities and management (thinning and fertilization) influence the potential of forest mixed plantations to mitigate (C sequestration) and adapt (stress resilience) to climate change, in a win-win approach. MFC project is funded by the LIFE PROGRAM within the European Union's funding instrument for the environment and climate action.

Mix for Change project will analyze in a common framework, and at unprecedented scale, synergies and trade-offs between the climate change mitigation and adaptation potential of mixed plantations and the fulfilment of stakeholders' objectives (Figure 1) and focuses focus on the following points:

I.   Characterize stakeholder's awareness of mixed plantations benefits and identify barriers to implementation under contrasting management objectives, biomes and cultural contexts.

II. Identify which type of species mixture optimize climate change mitigation on per biome basis (via C sequestration).

III. Characterize the resilience of mixed plantations to multiple climate change-related stresses.

Tony Chahine, The potential of Biodiversity for Adaptation Strategies to Global Change in Mediterranean Ecosystems. PhD thesis in Agrometeorology and Ecophysiology of Agricultural and Forest Systems, University of Sassari.

Figure 1. *Interdependencies of climate change-related stresses and management of forest plantations, and the way they are studied in the project's work packages (WPs). WP1 to WP3 address the ecological mechanisms underlying C sequestration (mitigation) and drought resilience (adaptation) in mixed forest plantations. WP4 addresses the stakeholders' management choices, as shaped by their objectives and motivations, and characterize the composition of existing forests. WP5 provides a synthesis by evaluating trade-off and synergies between adaptation, mitigation and stakeholders' objectives.*

IV.2.2   CAMBIO project

The CAMBIO is a project (https://www.cambio-treediversity.com) Led by the Department of Ecology and Biodiversity Conservation at the Faculty of Bioscience Engineering, Ghent University, Belgium, the CAMBIO project is a focused exploration of the potential for climate change mitigation and adaptation within young tree plantations. Through meticulous research and extensive collaboration, CAMBIO endeavors to identify specific tree species combinations that can enhance the functionality of planted forests in the context of a dynamically changing climate. The project's invaluable contributions are made possible through the generous funding and support provided by the BNP Paribas Foundation, as part of its Climate and Biodiversity Initiative. CAMBIO research initiative is primarily driven by three fundamental objectives:

Identification of Optimal Tree Species Mixtures: A primary goal of CAMBIO is to determine the most effective combinations of tree species that offer mitigation and adaptation strategies for the anticipated impacts of climate change.

Utilization of Scientific Insights: CAMBIO places a strong emphasis on the translation of scientific findings into tangible recommendations for the establishment of new experimental plantations and practical afforestation projects. The project leverages its scientific results to refine and enhance these guidelines, thereby facilitating the implementation of informed forest management strategies.

Broad Outreach and Impact: CAMBIO is committed to disseminating its findings across various strata of the community. This outreach includes engagement with practitioners through collaborative efforts with the Food and Agriculture Organization of the United Nations (FAO), and collaborations with major research groups affiliated with TreeDivNet. Furthermore, CAMBIO extends its reach to a wider audience through peer-to-peer networks and social media platforms.

In addition to its recommendations tailored for temperate regions, CAMBIO is dedicated to ensuring the applicability of its findings to tropical and sub-tropical countries. This endeavor is motivated by several critical factors:

i- High Biodiversity Hotspots: Tropical and sub-tropical regions host unparalleled levels of biodiversity, necessitating effective strategies for conservation and adaptation.

ii- Rapid Carbon Sequestration: The accelerated growth of plants in these regions contributes significantly to the removal of CO_2 from the atmosphere during the initial phases of forest establishment, making them pivotal in climate change mitigation efforts.

iii- Vulnerability of Local Populations: The inhabitants of tropical and sub-tropical areas are often the most susceptible to the adverse consequences of climate change, amplifying the urgency of effective adaptation measures.

IV.3 Enhancing Carbon Sequestration and Ecosystem Resilience in Mixed Forest Plantations

Mixed forest plantations are increasingly being recognized for their potential to mitigate climate change by sequestering carbon in plant biomass and soil. Studies have shown that mixed forest plantations can have a positive impact on root systems, hyphal production, and fungal communities, which play an important role in carbon sequestration (Luo et al., 2019; Peña et al., 2020). However, the effects of mixed forest plantations on carbon sequestration can vary depending on the diversity of tree species and the level of drought exposure. A study by Echeverria et al. (2019) found that mixed forest plantations with higher species diversity had higher carbon sequestration rates under dry conditions compared to monoculture plantations. Additionally, the study showed that mixed forest plantations with higher diversity had a greater ability to withstand drought stress, indicating the importance of diversity in maintaining ecosystem resilience. Furthermore, irrigation can also have a positive effect on photosynthetic performance and leaf traits in mixed forest plantations in Mediterranean climates (Martínez-Ferri et al., 2018). Understanding the complex interactions between plant species diversity, drought exposure, and irrigation in mixed forest plantations is critical for designing effective forest management strategies that maximize carbon sequestration while maintaining ecosystem functioning and resilience.

Forests play a critical role in mitigating climate change by sequestering carbon from the atmosphere. However, traditional monoculture plantations have limited ability to store carbon due to their low species diversity and lack of structural complexity. Mixed forest plantations, which consist of two or more tree species planted in close proximity, have emerged as a potential solution to enhance carbon sequestration and biodiversity conservation. Mixed forest plantations can support higher levels of biodiversity, provide greater resilience to climate change, and offer more diverse products for local communities. In recent years, research has focused on understanding the ecological performance and carbon sequestration potential of mixed forest plantations. Specifically, studies have examined the effects of mixed forest plantations on root systems, hyphal production, and fungal communities (Luo et al., 2019; Pena et al., 2020), as well as their variations in exposure and vulnerability to drought along diversity gradients (Echeverria et al., 2019). Moreover, irrigation can also play a crucial role in maintaining the photosynthetic performance and leaf traits of mixed forest plantations in

Mediterranean climates (Martínez-Ferri et al., 2018). This article provides an overview of the current understanding of the effects of mixed forest plantations on carbon sequestration,
as well as the key factors influencing their ecological performance, including tree species diversity, drought exposure, and irrigation.

Mixed forest plantations have been shown to enhance carbon sequestration by improving root systems, hyphal production, and fungal communities. According to research by Vicca et al. (2014), mixed forests tend to have higher root biomass and deeper root systems, which allow for greater carbon storage in soil. Mycorrhizal fungi are also important contributors to carbon sequestration in mixed forests, as they form symbiotic relationships with plant roots that improve nutrient uptake and facilitate carbon transfer to soil (Rillig et al., 2015). Furthermore, mycorrhizal fungi produce glomalin, a glycoprotein that increases soil carbon storage by binding with soil particles (Wu et al., 2019). Fungal communities in mixed forests are highly diverse and can also contribute to carbon sequestration, with studies showing that forest management practices that increase fungal diversity can enhance carbon storage in soil (Treseder et al., 2014). Overall, the root systems, hyphal production, and fungal communities in mixed forest plantations play a critical role in carbon sequestration and highlight the importance of sustainable forest management practices.

Mixed forest plantations are also known for their potential to enhance ecosystem resilience to drought events, but the effects of diversity on this relationship remain unclear. Research by Ruiz-Jaen and Potvin (2010) found that mixed species plantations had higher water use efficiency and were more resilient to drought than monoculture plantations. Additionally, mixed forests tend to have more diverse and complex root systems that can access deeper soil layers, allowing for greater access to water resources during drought events (Fischer et al., 2016). However, the relationship between diversity and drought response may not always be straightforward. Studies have shown that increasing diversity can also lead to higher variability in soil moisture, potentially increasing vulnerability to drought (Hartmann et al., 2014). Furthermore, the effects of diversity on drought response can vary depending on environmental conditions, such as soil type and precipitation patterns (Pretzsch et al., 2015). Overall, while mixed forest plantations can enhance ecosystem resilience to drought, the relationship between diversity and drought response is complex and requires further study.

Mixed forest plantations and irrigation can have significant effects on leaf traits and photosynthetic processes. A study by Martínez-Ferri et al. (2018) investigated the effects of mixed forest plantations and irrigation on leaf traits and chlorophyll a fluorescence in a Mediterranean climate. They found that mixed forest plantations led to higher values of maximum quantum yield of photosystem II (PSII) and higher values of photochemical quenching compared to monoculture plantations. Furthermore, irrigation had a positive effect on photosynthetic performance, increasing values of maximum PSII and non-photochemical quenching. However, mixed forest plantations and irrigation also led to changes in leaf traits, such as reduced specific leaf area and increased leaf thickness. These changes may have implications for carbon and water cycling in mixed forest plantations. Overall, the study highlights the potential benefits of mixed forest plantations and irrigation for enhancing photosynthetic performance, but also emphasizes the importance of considering the impacts on leaf traits and ecosystem functioning.

Previous studies have demonstrated that mixed forest plantations can have a positive impact on biodiversity, carbon sequestration, and soil quality (e.g., Montagnini and Nair, 2004; Puettmann et al., 2015). However, much of this research has been conducted in small-scale or observational studies, limiting our ability to draw broader conclusions about the performance of mixed forest plantations. The studies conducted in the experimental field provide a unique opportunity to investigate the impact of different plantation configurations on multiple ecosystem services and to compare the performance of different mixed forest systems under controlled conditions.

Briefly, diverse forests have been shown to fix more atmospheric carbon, store more carbon above and belowground, balance the evapotranspiration and the transpiration, decrease the likelihood and severity of fires and pest outbreaks, and mitigate microclimatic conditions under climate change (Figure 2).
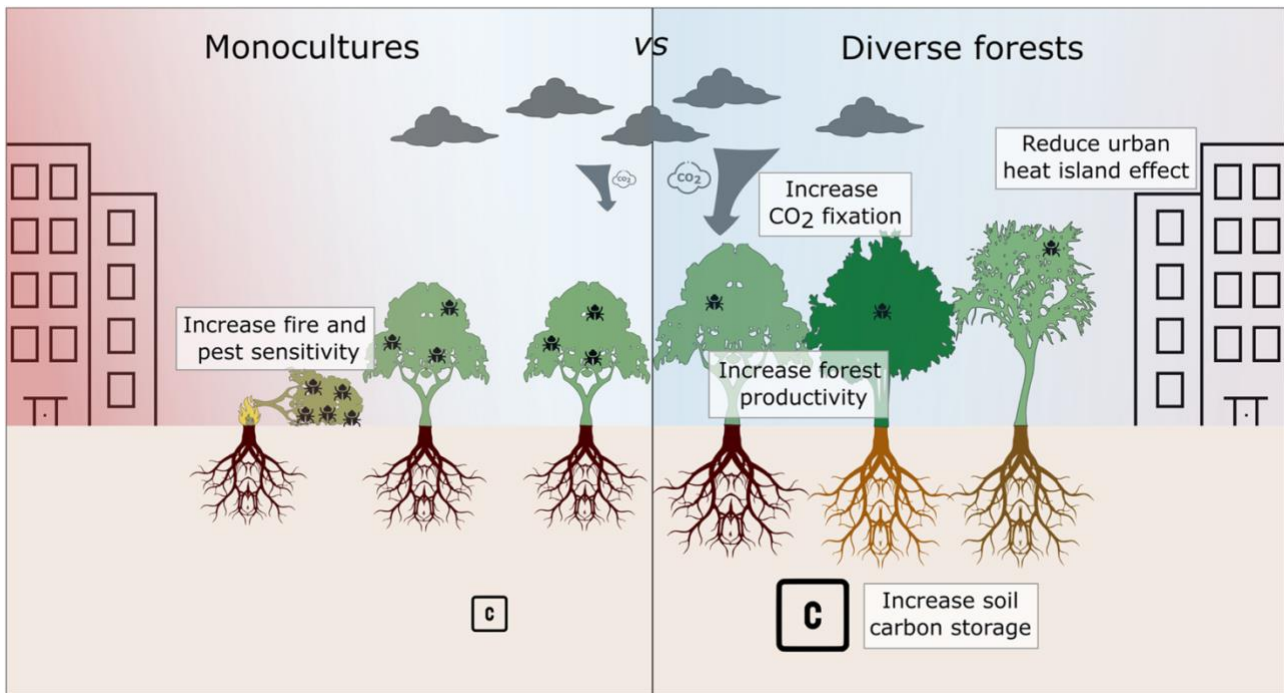
Figure 2. *Conceptual figure of the effects of tree diversity on ecosystem properties related to climate change mitigation (source: Beugnon et al., 2021).*

All studies were conducted in an experimental field located in Sardinia "IDENT-MACOMER The area of the field is 0.6 hectares and contains multiple plots planted with different configurations of mixed forest plantations in order to provide insights into the design and management of sustainable mixed forest plantations.
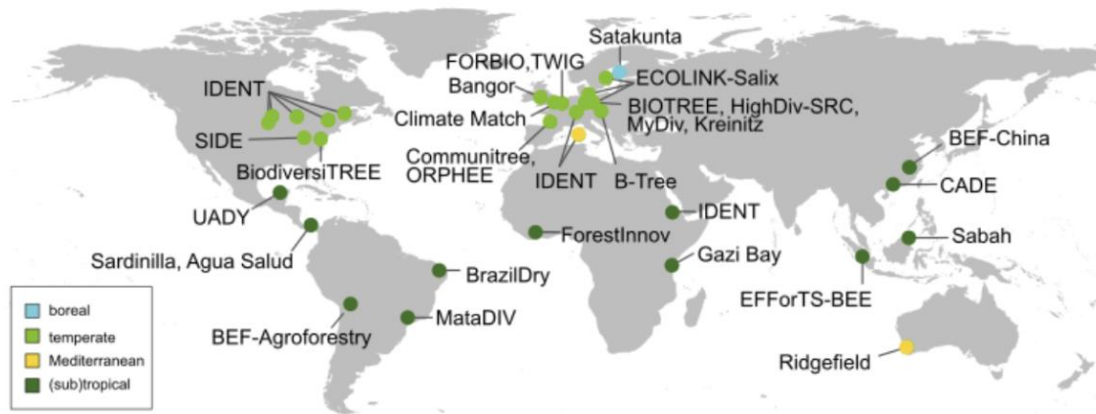
IV.3.1   Tree diversity experiments

"TreeDivNet" is a global network of tree diversity experiments that offers unique opportunities for (1) research on the relation between tree species diversity and ecosystem functioning in major forest types around the world and (2) coordinated knowledge transfer towards forest policy and management.

There are currently at least 25 tree diversity experiments exploring the functional consequences of tree species mixing. Similar as in the conventional model systems, they encompass different species compositions in a replicated and semi-randomized design, with the inclusion of all monocultures as well as 2, 3, 4 or 6 species in mixture. Tree diversity experiments cover a total area of 821 hectares in different climates (Figure 2). They have been developed through independent initiatives, but they participate in a global platform network (TreeDivNet) where synthesis work is assisted and field expertise is shared (Verheyen et al. 2015). Close affinity with common silvicultural practices is

107

generally objected, but still, experiments differ remarkably in terms of diversity index manipulated (species richness SR, functional diversity, phylogenetic diversity, genetic diversity and evenness), upper-diversity level of species richness (from 2 to 18 species), type of tree species mixing (either patch planting or individual-based planting), spatial scale (plots sizes ranging from 0.24 to 12 000 m2), planting density (between 40 cm and 300 cm, but usually constant within experiments), site heterogeneity (e.g. from flat sites to hilly slopes) and more (Verheyen et al. 2015). Only 13 experiments were established before 2010.

"IDENT-MACOMER" is part of the International Diversity Experiment Network with Trees (IDENT), Tobner et al. 2014) within the (TreeDivNet) network.

Figure 3. *Global distribution of tree diversity network experiments contributing to TreeDIvNet (www.treedivnet.ugent.be). TreeDivNet offers a science-based research platform for discovering the relationships between tree species diversity and ecosystem functioning through experimental approaches (Verheyen et al. 2015). The 25 experiments cover dominant ecoregions, including Boreal, Temperate, Mediterranean, and Sub-tropics. Because of their independent establishment, plantations use various design alternatives and their age ranges between 1 to 19 years old. Source: www.treedivnet.ugent.be*

IV.4 Materials and Methods

IV.4.1   Study site

IDENT-MACOMER is situated in Macomer on the island of Sardinia in Italy, within the "Saint Antonio - Sardinian Forest Authority" nursery and is part of the International Diversity Experiment Network with Trees (IDENT) at an altitude of 640 meters above sea level. The local climate is classified as hot-summer Mediterranean (Köppen: Csa) with warm and dry summers and mild winters featuring moderate rainfall. Monthly average temperatures at the plantation site range from 6.5 °C (January) to 23.9 °C (August), while monthly rainfall ranges from 135 mm (December) to 7 mm (July), with an accumulated rainfall of 905 mm annually (Ente Autonomo Flumendosa, 1998). The area was previously used as a tree nursery for local reforestation efforts until 2008, after which the fields were left unmanaged, but grasses were occasionally removed. A meteorological station was installed adjacent to the experiment in 2015 and measures precipitation, photosynthetic active radiation, wind speed and direction, air temperature, and relative humidity. The station has sensors placed at various heights and records data every 5 minutes. The experimental design features hierarchically organized trees distributed over 308 plots and seven blocks (Figure 4), with each block consisting of 44 plots of 3.2 m by 3.2 m. Each plot has 64 seedlings planted at 40 cm intervals. All blocks were irrigated during the dry season in the first year (2014), and three selected blocks were irrigated with 20 mm every 15 days from June to September in subsequent years using a pipe system. The remaining four blocks were not irrigated and serve as controls. Species selection was based on site requirements and considering local availability of seedlings in the nursery.

In total, 12 native woody species (Table 1) were selected for the experiment, including both shrubs (three species) and trees (nine species), with three conifers (*Pinus halepensis* Mill*., Pinus pinaster* Ait*.,* and *Pinus pinea* L.), six evergreen broad-leaved species, and three deciduous broad-leaved species (*Acer monspessulanum* L*., Arbutus unedo* L*., Fraxinus ornus* L*., Olea europea* L*., Phillyrea latifolia* L*., Pistacia lentiscus* L*., Quercus ilex* L*., Quercus pubescens* Willd*.,* and *Quercus suber* L*.).*
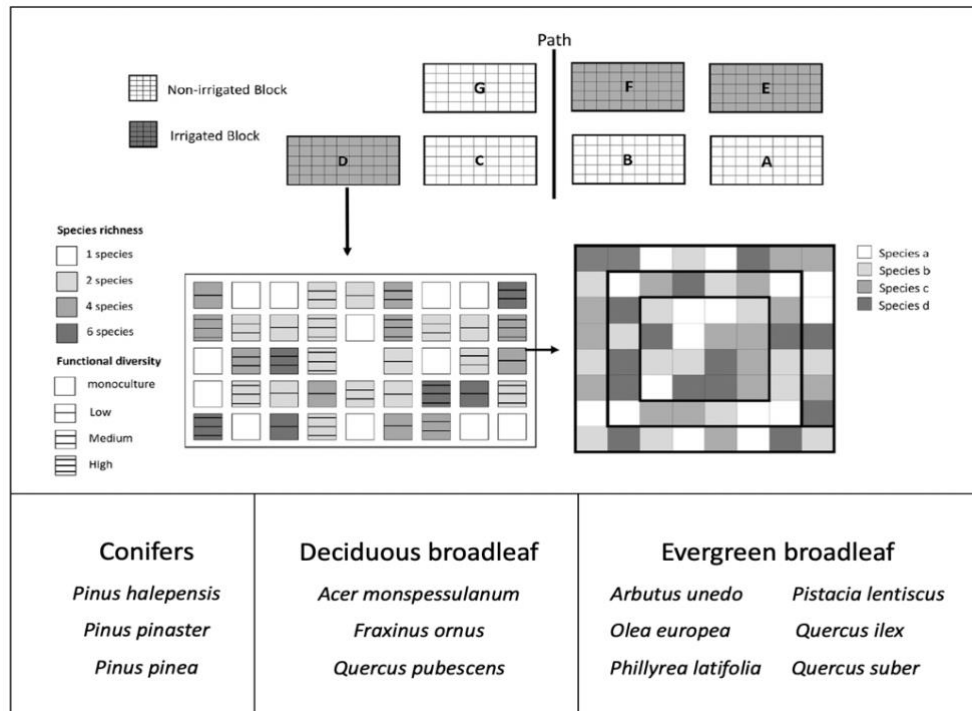
Table 1. *Taxonomic Classification, Common Nomenclature, and Leaf Habit Characteristics of Studied Tree Species (IDENT-MACOMER).*

Latin name	Common name	Leaf habit
Quercus ilex L.	Holm Oak	Evergreen
Quercus suber L.	Cork Oak	Evergreen
Quercus pubescens Willd.	Downy Oak	Deciduous (Marcescent)
Pinus pinaster Ait.	Maritime Pine	Evergreen
Pinus pinea L.	Stone Pine	Evergreen
Pinus halepensis Mill.	Aleppo Pine	Evergreen
Phillyrea latifolia L.	Green Olive Tree	Evergreen
Acer monspessulanum L.	Montpellier Maple	Deciduous
Pistacia lentiscus L.	Mastic Tree	Evergreen
Arbutus unedo L.	Strawberry Tree	Evergreen
Fraxinus ornus L.	Manna Ash	Deciduous
Olea europea L.	Olive Tree	Evergreen

IV.4.2   Experimental design

The experimental design includes a first diversity gradient created by manipulating species richness at four levels and a second gradient created by manipulating functional diversity (FD), which is orthogonal (independent) to the first gradient.

Figure 4. *Experimental design of IDENT-MACOMER (Sardinia, Macomer), a single-site tree diversity experiment created in 2014. The experiment is hinges on a high-density planting design (0.4 m by 0.4 m) and small plot sizes (10.2 m2). The planting corresponds to densities of regenerating forests and should speed-up the onset of tree-tree interactions (Tobner et al. 2014). The small plot size allows more plot replicates, although it is at the cost of a plot's scientific lifespan. On top of the figure, a site plan is shown with three irrigated (grey) and four non-irrigated (white) blocks, which allows testing the stress gradient hypothesis. In the middle left of the figure, the disposition of 44 plots within a block, including orthogonal gradients of SR and FD is shown; In the middle-right there is the arrangement of 64 plants in a four-species plot. Bold lines represent inner, middle and outer frame. Species relative abundances within these frames are alike and the outer frame was not included in the analyses to avoid plot edge effects. A broad species pool of 12 site-adapted Mediterranean species (including conifers, deciduous broadleaved and evergreen broadleaved species) was used to create the monocultures and mixed communities (lower part). All plots were regularly weeded in the first three years to avoid overgrow by herbaceous vegetation.*

IV.4.3   Experiments

Three separate experiments were conducted on the site, with each experiment consisting of a randomized complete block design with three replicates as follows:

IV.4.3.1 Experiment 1

The first experiment entitled Mixed Forest Plantations: Effects on Roots, Hyphal Production and Fungal Communities, and their Role in Carbon Sequestration The study is led by Ramona Werner, Hans Sandén, and Agnès Robin from the Institute of Forest Ecology, Department of Forest and Soil Sciences at the University of Natural Resources and Life Sciences (BOKU) in Vienna, Austria, and CIRAD, UMR EcoandSols in Montpellier, France.

In this study, two other experimental fields were utilized (B-Tree Austria and ORPHEE in France). The study investigated the effects of mixed forest plantations on root systems, hyphal production, and fungal communities, and their contribution to carbon sequestration. The experiment used mycorrhizal in-growth bags filled with sand to measure mycorrhizal biomass production in mixed stands of different tree species.

Triangular in-growth bags (Figure 5) with dimensions of 7 x 7 cm were used to investigate fungal biomass in the soil. These bags were filled with 13 g of acid-washed quartz sand, with a mesh size of 50 μm, and were placed at a depth of 8 cm at the beginning of the growing season. The in-growth bags were randomly placed in all VIP plots, where different combinations of tree species were sampled in each mixture. After six months, the bags were visually examined for ingrown hyphae, and Ergosterol measurements are currently underway to estimate fungal biomass.

Preliminary results showed a strong effect of site and species-specific soil parameters, but no general effect of increased diversity on fungal hyphae colonization. Ergosterol measurements for estimation of fungal biomass are still in progress.

Figure 5. *Mycorrhiza in-growth bags were used in IDENT-MACOMER.*

IV.4.3.2 Experiment 2

The second experiment entitled variations in exposure and vulnerability to drought along diversity gradients at IDENT-MACOMER. The study is led by Renaud Decarsin from the National Institute of Agricultural Research (INRA), Avignon, France.

The study investigated the variations in exposure and vulnerability to drought along diversity gradients in mixed forest plantations.

This study examines how forest ecosystems are experiencing more frequent and intense droughts, which threaten tree survival and forest functions. To increase resistance to drought, tree diversity is often considered, but the mechanisms behind this are not well understood.

Through this study the purpose is to assesses the potential changes in drought resistance of various tree species planted along diversity gradients (from monoculture to 5 species mixture) in five experiments across Europe, the focus of our discussion will center around IDENT-MACOMER.

The study intends to quantifies the hydraulic safety margin, which is the difference between exposure to stress and the vulnerability to stress, to understand if drought resistance changes with diversity and if those changes are related to exposure or vulnerability.

The results will offer valuable information for policy makers, practitioners and researchers and reflect new perceptions to help decision-makers in restoration and afforestation programs.

The study aims to observe variations in hydraulic safety margins (HSM) based on species richness and composition in mixed plantations, and whether these vary with drought strategies. Comparisons between geographical regions will also be made. However, the study is limited by the fact that the sites were chosen by managers within the Mix for Change project framework, and species present vary across sites, which makes inter-site comparisons difficult and may require presumptions or inferences. Nevertheless, each site is a validated experimental device that can be analyzed independently. The stratification of a mixed plantation's tree could limit water stress exposure through its impact on microclimate. The species' variable architecture and differentiated growth could lead to differentiated shading of certain species' trees, reducing evapotranspiration during intense droughts. This would limit sap flow and tension on the xylem, decreasing soil water reserves differently than in a monospecific plantation. Hydraulic safety margins (HSM) account for the range of stresses a species can sustain in a given environment. In 2022, physiological traits were measured at four European sites to characterize HSM along diversity gradients.

The aim is to measure exposure (base and minimum Psi, native Pi, Pi0, PiTlp) and vulnerability to drought (P50) for the following species:

Table 2. *Plant Compositions in Monoculture, Intermediate Mix, and High Mix Plots*

Compositions of monoculture	Compositions of plots with intermediate level mixture	Compositions of plots with high mixture level
Pipe (*Pinu pinnaster*)	Phla-Fror; Arn-Acmo; Acmo-Qupu; Pipe-Fror	Pipe-Fror-Acmo-Qupu
Fror (*Fraxinu ornus*)		
Qupu (*Quercus pubescens* Willd.)		
Acmo (*Acer monspessulanum* L.)		
Arun (*Arbutus unedo* L.)		
Phla (*Phillyrea latifolia* L.)		

Table 3. *The table above represents a brief description of the measure and its relevance, and the periods envisaged for their implementations.*

Measure	Principle	Interest	Period concerned
Vulnerability to cavitation (P50)	Draw a hydraulic conductance loss curve as a function of a voltage imposed by a centrifuge (the cavitron)	Rapid determination of the P50, a trait used in the calculation of HSMs	From 30/05 to 10/07 - 2022
Basic water potential (Psibase)	Measure the internal tension at the xylem by placing a sample (leaf or branch apex) in a chamber and increasing the pressure until the flow of evapotranspiration reverses and internal water emerges through the stem tip protruding through a seal in the chamber.	Index of plant exposure to soil water stress.	During a significant drought -> during the months of July and August
Midday water potential (Psimin)		Index of plant exposure to soil water stress and microclimate.	
Native osmotic potential (Pi)	To measure the amount of osmotically active molecules in chlorophyll cells. Allowing the calculation of a pressure related to the exposure to water stress.	Access to a complementary index of exposure to water stress.	
Osmotic potential at full turgor (Pi0)		Access to a vulnerability index (Possibility of finding the potential for turgidity loss)	

The table above represents a brief description of the measure and its relevance, and the periods envisaged for their implementations.

It provides information on five measures related to plant water potential and osmotic potential. The first column lists the measures as follows: Vulnerability to cavitation (P50), Basic water potential (Psibase), Midday water potential (Psimin), Native osmotic potential (Pi), and Osmotic potential at full turgor (Pi0). The second column describes the principle behind each measure, which includes drawing a hydraulic conductance loss curve, measuring internal tension at the xylem, and measuring the amount of osmotically active molecules in chlorophyll cells. The third column outlines the interest of each measure and the periods envisaged for their implementation.

The third column provides more detailed information on the relevance of each measure. The first cell describes the use of the rapid determination of the P50 as a trait in the calculation of hydraulic safety margins (HSMs), which is related to plant drought tolerance. The second cell corresponds to an index of plant exposure to soil water stress, which is calculated based on the second and third cells of the first column. The third cell is an index of plant exposure to soil water stress and microclimate, which corresponds to the third cell of the first column and the second cell of the second column. The fourth cell provides access to a complementary index of exposure to water stress, which matches the fourth cell of the first column and the third cell of the second column. The fifth cell provides access to a vulnerability index, which measures the possibility of finding the potential for turgidity loss, and corresponds to the fifth cell of the first column and the third cell of the second column.

As preliminary results in the IDENT-MACOMER site showed that hydraulic safety margins (HSM) tended to increase with higher species richness, but this relationship was stronger in the drought-prone treatment than in the control treatment. This suggests that mixed plantations with higher species richness may have a greater ability to maintain water transport under stressful conditions. Additionally, the study found that HSM tended to decrease with increasing vulnerability to cavitation. This means that species that are more vulnerable to cavitation may have lower HSM and be more susceptible to hydraulic failure under drought conditions. Overall, these results highlight the importance of species diversity and vulnerability to cavitation in maintaining hydraulic safety in mixed plantations under drought conditions.

IV.4.3.3 Experiment 3

The third experiment entitled "Effect of tree species mixing and irrigation on chlorophyll, fluorescence and leaf traits in a Mediterranean climate", Sardinia, Italy is led by Abebe Damtew Awraris and Bart Muys from the Faculty of Bioscience Engineering, Department of Earth and Environmental Science, Division of Forest, Nature, and Landscape at Leuven, Belgium.

Chlorophyll a fluorescence and Surface Leaf Area (SLA) measurement

In this experiment, the analysis of chlorophyll *a* fluorescence only focused monocultures and mixtures composed of two species. Three species were selected from the center of the plot, thus excluding the trees planted in the outermost rows to avoid borderer effects.
From different sections of the crown, small branches with several leaves or needles were collected from the upper canopy (Sun leaves), co-dominant (semi-shaded leaves), and lower canopy (shaded leaves) using extension loppers (Figure 6).

The height of the dominant and co-dominant species as well as the height of the lowest crown portion were assessed with a metric pole.
The incoming radiation was measured above the canopy and at the height where leaves were sampled to asses an extinction coefficient based on the ratio of the two measurements and this ratio was used a s a proxy of the light environment.
Part of the leaves were used to assess chl a fluorescence while another part was used to asses the Specific leaf area. 10 leaves were numbered, scanned and dried in an oven at 60 °C for three days after which they were weighted. The area of each leaf was assessed from the scans using ImageJ software.

Leaves were collected at night (from 11:00pm to 5:00am) and brought to a nearby (100m) housing where they were kept in the dark and at 5 °C before measuring. Chl a passive fluorescence was assessed with a HandyPEA fluorimeter (Hansatech Instruments Ltd., Pentney, Norfolk, UK). Fluorescence rise OJIP curves were induced by 1 s pulses of red light (650 nm, 3500 µmol m-2 s -1). The fluorescence transients show a polyphasic shape, where O refers to the initial fluorescence level, K (300 µs), J (2 to 3 ms) and I (30 ms) are intermediate levels of the fluorescence emission, and P (500-800 ms - 1s) is the peak level of fluorescence. The latter indicates the highest, or maximal, fluorescence intensity (FM) when saturating light is applied to the leaf. Of the high number of

parameters yielded by the analysis of the OJIP curve, the parameters selected were FV/FM, i.e. the ratio between variable and maximal fluorescence [FV/FM =(FM-F0)/FM], that expresses the maximum quantum yield of primary photochemistry, and ΔVIP, or I-P phase [ΔVIP = 1-VI] i.e. the amplitude of the relative contribution of the I-to-P rise to the OJIP transient. These parameters are connected respectively to the processes of photon capture and first photochemical events of PSII, and to the efficiency of the electron transport around the photosystem I (thermal phase). FV/FM and ΔVIP, represent respectively the overall efficiency of PSII and PSI (Schansker et al., 2005; Oukarroum et al., 2009), and are considered suitable for the screening of a great number of samples in large scale ecological studies Bussotti et al. (2020).
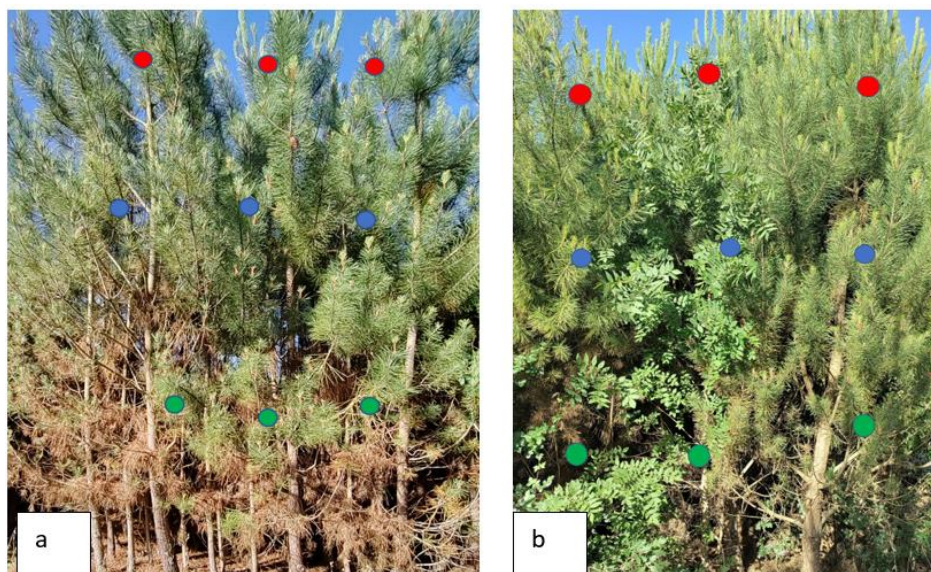
Figure 6. *Location of sample leaves or needles from monoculture (a) and two-species mixture (b) for chlorophyll fluorescence analysis. Sun leaves, fully exposed leaves are shown in red, partially shaded leaves are shown in blue, and entirely shaded leaves (elucidated in green color).*

IV.4.4   Statistical analysis

The leaves and needles of 12 tree and shrub species were assessed for chlorophyll fluorescence (ChlF) parameters at the sapling stage in 2018 and the tree stage in 2022. Then, the Linear Mixed Model (LMM) analysis were ran to evaluate whether Irrigation, Species richness, species identity, functional diversity, Light level, species composition, and their interaction predicted ChlF parameters at both sapling and tree stages using an R Package software version 4.2.2.

IV.4.5   Results

Chlorophyll Fluorescence analysis at sapling stage

The study found that the maximum photochemical efficiency and electron transfer efficiency of saplings were associated with species identity, functional diversity, and species composition. The efficiency generally decreased with functional diversity, and the lowest electron transfer efficiency was found in *Acer monspessulanum* L.. Irrigation did not have a significant impact on the efficiency indexes.

The study of saplings, the mean maximum photochemical efficiency FV/FM (φPo), an indicator of overall photosynthetic potential, was 0.734 ± 0.018 SE. The maximum photochemical efficiency was significantly associated with species identity, functional diversity, and species composition. The highest quantum yield was observed in *Pistacia lentiscus* L.*, Quercus pubescens* Willd.*,* and *Arbutus unedo* L., while the lowest was observed in *Fraxinus ornus* L. and *Quercus ilex* L. The efficiency of electron transfer from primary to secondary acceptors of PSII (ψEo) showed a significant relationship with functional diversity and species composition. The efficiency with which an electron can be transmitted from PSII secondary acceptor to PSI primary acceptors (δRo) showed a significant negative relationship with species richness and functional diversity.

The photochemical and electron transfer efficiency of saplings is associated with species identity, functional diversity, and species composition. Irrigation did not have a significant impact on efficiency indexes. The density of active reaction centers is significantly associated with species identity, species richness, and functional diversity. The highest and lowest density of active reaction centers are found in *Quercus pubescens* Willd. and *Fraxinus ornus* L., respectively. The performance indexes (PI$_{ABS}$) and (PI$_{TOT}$) were significantly related to species identity, species richness, and functional diversity.

The study found that the density of active reaction centers (RC/ABS) in saplings is associated with species identity, species richness, and functional diversity. Monoculture and low functional diversity showed higher (RC/ABS) density. *Quercus pubescens* Willd. has the highest density, while *Fraxinus ornus* L. has the lowest. Specific energy fluxes, such as absorption flux (ABS/RC), trapped energy flux (TRo/RC), electron transport flux (ETo/RC), and dissipated energy flux (DIo/RC) per active reaction center, were significantly associated with species richness, species identity, and functional diversity. Performance indexes (PI$_{ABS}$) and (PI$_{TOT}$) preseted in table 3 were significantly related to

species identity, species richness, and functional diversity. *Quercus suber* L. performed better in both indexes, while *Fraxinus ornus* L. showed a drastic decrease in overall performance.

Table 4. *Performance Index Definitions: The table outlines the formulas and descriptions for two photosynthetic performance indexes, PIABS and PITOT, which assess energy conservation from photon absorption by PSII to the reduction of intersystem electron acceptors and photosystems I end-acceptors, respectively.*

Performance indexes	
PI_{ABS}	The performance index for energy conservation from photons absorbed by PSII until the reduction of intersystem electron acceptors. (RC/ABS) [φPo / (1-φPo)] [ΨEo /(1- ΨEo)]
PI_{TOT}	The performance index for energy conservation from the photons absorbed by the photosystems II to the reduction of photosystems I end-acceptors. PIABS [$\delta R0$ / (1-$\delta R0$)]

Chlorophyll Fluorescence Analysis at the tree stage

This paragraph discusses the results of a study on the efficiency of photosynthesis and electron transfer in trees. The study found that the efficiency of primary photochemistry was significantly related to irrigation, species richness, species identity, light level, and their interaction. Maximum quantum yield was higher in irrigated, mixed stand, and semi-shaded conditions. However, the efficiency declined when irrigation was applied in a mixed stand. The efficiency of electron transfer was also significantly associated with irrigation, species richness, species identity, and light level, with *Pinus pinaster* Ait. having the highest efficiency. The efficiency of electron transfer to the PSI end acceptors was significantly higher in monoculture, low functional diversity, and sunny conditions.

The density of active reaction centers in trees is related to species identity, irrigation, species richness, and functional diversity. Irrigation, monoculture, low functional diversity, and low light levels lead to higher active reaction center density. *Quercus pubescens* Willd. has the highest density of active reaction centers, and *Pinus pinea* L. has the lowest. Irrigation, species mixture, and sunny conditions significantly increase the density of active reaction centers. PIABS and PITOT) significantly improve with irrigation, species richness, and light level, but drop with increased functional diversity. *Pinus pinaster* Ait. has the highest PIABS and PITOT values, while *Pistacia lentiscus* L. has the lowest.

IV.5 Results

The recent studies conducted in "IDENT-MACOMER" have shed light on the mechanisms by which mixed forest plantations contribute to carbon sequestration and enhance ecosystem resilience. One such study examined the effects of mixed forest plantations on roots, hyphal production, and fungal communities, and their role in carbon sequestration. The study revealed that mixed forest plantations can enhance soil carbon sequestration by increasing the abundance and diversity of fungal communities, which play a crucial role in nutrient cycling and carbon storage.

Another study investigated the variations in exposure and vulnerability to drought along diversity gradients in mixed forest plantations. The results showed that increasing species diversity in mixed forest plantations can enhance the resilience of trees to drought, by improving water-use efficiency and reducing water loss.

A third study examined the effect of mixed forest plantations and irrigation on chlorophyll a fluorescence and leaf traits in a Mediterranean climate. The study found that mixed forest plantations can enhance photosynthetic efficiency and reduce water stress in trees, particularly under drought conditions.

Taken together, these studies highlight the potential of mixed forest plantations as a strategy for enhancing the ecological and economic benefits of forests while promoting their resilience to climate change. In this article, we will review these recent findings and discuss their implications for the design and management of sustainable mixed forest plantations.

References

Beugnon R, Ladouceur E, Sünnemann M, Cesarz S, Eisenhauer N. (2021). Diverse forests are cool: promoting diverse forests to mitigate carbon emissions and climate change.
J Sustain Agric Environ. 1:5–8. https://doi.org/10.1002/sae2.12005

Contributions of a global network of tree diversity experiments to sustainable forest plantations. Ambio 45, 29–41.

Depauw, L., De Lombaerde, E., Dhiedt, E., Blondeel, H., Verheyen, K., Baeten, L., et al. (2024). Enhancing Tree Performance Through Species Mixing: Review of a Quarter-Century of TreeDivNet Experiments Reveals Research Gaps and Practical Insights. *Current Forestry Reports*. https://doi.org/10.1007/s40725-023-00208-y

Echeverria, C., Newton, A. C., Lara, A., Fuentes-Castillo, T., Armesto, J. J., and Pérez, C. A. (2019). Tree diversity enhances carbon storage and sequestration in a drought-prone mixed temperate forest. Ecological Applications, 29(6), e01918.

Fischer, R., Bauhus, J., Böhm, C., Fischer, A., Häberle, K.H., Huss, J., and Rötzer, T. (2016). Lessons learned from mixed forests. In Mixed-species forests (pp. 399-432). Springer International Publishing.

Hartmann, H., Moura, C.F., Anderegg, W.R., Ruehr, N.K., Salmon, Y., Allen, C.D., and Klein, T. (2014). Research frontiers for improving our understanding of drought-induced tree and forest mortality. New Phytologist, 206(2), 329-333.

Luo, X., Chen, J., Yu, S., Wang, H., and Luo, Y. (2019). Effects of mixed forest plantations on carbon sequestration: a review. Forests, 10(3), 246.

Martínez-Ferri, E., Carrión, V., de la Riva, J., and Escudero, A. (2018). Effect of mixed plantations and irrigation on chlorophyll a fluorescence and leaf traits in a Mediterranean climate. Photosynthetica, 56(4), 1237-1246.

Montagnini, F., and Nair, P. K. R. (2004). Carbon sequestration: An underexploited environmental benefit of agroforestry systems. Journal of Agroforestry Systems, 61(1-3), 281-295.

Peña, C. L., Díaz-Rua, R., Pérez-Ramos, I. M., Villar, R., and Marañón, T. (2020). Fine roots and hyphal production in mixed forest plantations: the role of tree diversity, composition and traits. Plant and Soil, 448(1-2), 357-373.

Pretzsch, H., Biber, P., Schütze, G., Uhl, E., and Rötzer, T. (2015). Forest stand growth dynamics in Central Europe have accelerated since 1870. Nature Communications, 6, 1-10.

Puettmann, K. J., Wilson, S. M., Baker, S. C., Donoso, P. J., Drössler, L., Amente, G., and Knoke, T. (2015). Silvicultural alternatives to conventional even-aged forest management—what limits global adoption?. Forests, 6(5), 1668-1696.

Ruiz-Jaen, M.C., and Potvin, C. (2010). Can we predict carbon stocks in tropical ecosystems from tree diversity? Comparing species and functional diversity in a plantation and a natural forest. New Phytologist, 189(4), 978-987.

Rillig, M.C., Wright, S.F., Shaw, M.R., and Field, C.B. (2015). Artificial selection for fungal mutualists facilitates climate change adaptation. Proceedings of the Royal Society B: Biological Sciences, 282(1814), 20150268.

Tobner, C.M., Paquette, A., Reich, P.B., Gravel, D. and Messier, C. (2014). Advancing biodiversity–ecosystem functioning science using high-density tree-based experiments over functional diversity gradients. Oecologia, 174(3): 609-621.

Treseder, K.K., Balser, T.C., Bradford, M.A., Brodie, E.L., Dubinsky, E.A., Eviner, V.T., and Wallenstein, M.D. (2014). Integrating microbial ecology into ecosystem models: challenges and priorities. Biogeochemistry, 118(1-3), 1-9.

Verheyen, K., Vanhellemont, M., Auge, H., Baeten, L., Baraloto, C., Barsoum, N., Bilodeau Gauthier, S., Bruelheide, H., Castagneyrol, B., Godbold, D., Haase, J., Hector, A., Jactel, H.,

Koricheva, J., Loreau, M., Mereu, S., Messier, C., Muys, B., Nolet, P., Paquette, A., Parker, J., Perring, M., Ponette, Q., Potvin, C., Reich, P., Smith, A., Weih, M., and Scherer-Lorenzen, M. (2015).

Vicca, S., Bahn, M., Estiarte, M., van Loon, E.E., Vargas, R., Alberti, G., and Peñuelas, J. (2014). Can current moisture responses predict soil CO2 efflux under altered precipitation regimes? A synthesis of manipulation experiments. Biogeosciences, 11(12), 3307-3327.

Wu, L., Chen, J., Jiang, Y., Zhang, X., Chen, Y., Wei, G., and Zhang, Y. (2019). Glomalin-related soil protein contributes to soil carbon storage in a subtropical forest. Soil Biology and Biochemistry, 130, 151-160.

Tony Chahine, The potential of Biodiversity for Adaptation Strategies to Global Change in Mediterranean Ecosystems. PhD thesis in Agrometeorology and Ecophysiology of Agricultural and Forest Systems, University of Sassari.

V. Conclusion

The multifaceted findings of this study have advanced our understanding of the complex dynamics within mixed forest ecosystems, shedding light on their implications for ecosystem management and conservation strategies.

One of the striking outcomes of this research pertains to the potential interaction or facilitation between pine trees and herbivorous insects. It was observed that shaded leaves in the presence of pine trees tend to be thinner, softer, and richer in water content, making them more attractive to herbivorous insects. Furthermore, the microenvironment beneath the pine tree canopy, characterized by reduced temperature fluctuations, increased air humidity, and decreased radiation levels, provides a favorable habitat for these insects. These intriguing findings call for further investigations to uncover the underlying mechanisms that drive these interactions and to better understand their ramifications for mixed forest ecosystems.

The dynamics governing herbivory patterns in these ecosystems are multifaceted and influenced by an array of factors. These factors include species interactions, competitive dynamics, as well as environmental parameters such as microclimates and soil conditions. A comprehensive understanding of these complex interactions is imperative for clarifying the observed variations in herbivory and for devising effective strategies for the sustainable management and preservation of mixed forests. To this end, further research is warranted to delve deeper into the ecological and chemical mechanisms at play, enhancing our comprehension of this intricate ecological interplay.

Moreover, this study has uncovered the remarkable productivity benefits of tree species mixing, particularly in Mediterranean contexts. The positive relationships observed between tree diversity and above ground biomass accumulation bolster the overarching theory of positive Biodiversity and Ecosystem Function relationships. These findings underscore that, on average, mixed-species forest plantations outperform monocultures, underlining the ecological and economic advantages of diversified forest management, particularly in the fight against climate change and the enhancement of ecosystem resilience.

Additionally, this research has challenged a widely held notion regarding the relationship between water constraints and Biodiversity and Ecosystem Function relationships, as postulated by the Stress

125

Gradient Hypothesis (SGH). Contrary to SGH, the study has demonstrated that positive BEF relationships are higher at higher water availability but that the positive BEF relationship increase with FDis independently of the water availability. This insight still positions mixed forest plantations as a robust adaptation and mitigation strategy for climate change, but also suggests that with increasing droughts the benefits of diversity for ecosystem functions will decrease independently of SR or FDis and for now, we could not identify mixes that are likely to counteract the impacts of climate change.

The recent studies conducted within the "IDENT-MACOMER" project added some insights on the underlying mechanisms that drive the contributions of mixed forest plantations to carbon sequestration and ecosystem resilience. These findings highlight that increasing species diversity within mixed forest plantations can enhance tree resilience to drought, improving water-use efficiency and reducing water loss. Finally, mixed forest plantations have been shown to enhance photosynthetic efficiency and reduce water stress in trees, particularly under drought conditions. Both preliminary findings are coherent with the results found in the first two chapters.

In conclusion, the research presented in this thesis represents a significant contribution to the field of ecological studies, particularly in the context of mixed forest ecosystems and their response to changing environmental conditions. The insights gained from this study provide a robust scientific foundation for promoting mixed-species forest plantations as nature-based solutions to address the causes and consequences of climate change. Additionally, this work emphasizes the importance of considering tree species composition in ecosystem management and highlights the ecological, economic, and climate resilience benefits of mixed forest plantations.

Moving forward, further research is encouraged to deepen our mechanistic understanding of these complex relationships and to facilitate the generalization of experimental findings. This knowledge will be instrumental in guiding forest management practices, promoting sustainable land use, and ensuring the conservation of biodiversity in the face of global environmental challenges. Moreover, the findings of this study have practical implications for forest management and land-use planning, suggesting that mixed-species forest plantations can be an effective strategy for enhancing both the ecological and economic benefits of forests while promoting their resilience to climate change. These insights should inform policies and practices aimed at achieving sustainable and resilient ecosystems in the face of ongoing environmental challenges.